Malaria and helminth co-infections in school and pre-school children in Magu district, Tanzania

Kinung'hi, Safari Methusela

Publication date:
2011

Document version
Publisher's PDF, also known as Version of record

Ph.d. thesis

Name of Department: Department of Veterinary Disease Biology

Name of section: DBL – Centre for Health Research and Development, Faculty of Life Sciences, University of Copenhagen, Denmark

National Institute for Medical Research (NIMR),
Mwanza Medical Research Centre, Mwanza, Tanzania

Author: Safari Methusela Kinung'hi

Title: Malaria and Helminth Co-infections in School and Pre-school Children in Magu District, Tanzania

Subject: Malaria and Helminth Co-infections in School and Pre-school Children in Magu District, Tanzania

Supervisor: Dr. Pascal Magnussen, DBL, Department of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen, Denmark

Prof. Birgitte J. Vennervald, DBL, Department of Veterinary Disease Biology Faculty of Life Sciences, University of Copenhagen, Denmark

Dr. Nicholas J. S. Lwambo, National Institute for Medical Research (NIMR), Mwanza Medical Research Centre, Mwanza, Tanzania

Dedication

This thesis work is dedicated to my wife Esther Innocent and our three children Noel, Frank and Emmanoela. Also to my father, the late Methusela Kinung'hi, my mother Minza Mhameji and my brothers and sisters. Their love and support was a driving force towards the accomplishment of this research work.

TABLE OF CONTENTS Page

Acknowledgements

I would like to convey my sincere gratitude to all those who in one way or another facilitated the planning and implementation of this study. Special thanks go to my supervisors Dr. Pascal Magnussen, Professor Birgitte J. Vennervald and Dr. Nicholas J. S. Lwambo for their continued guidance and support throughout the duration of the study. I extend my sincere thanks to the Management of Mwanza Medical Research Centre particularly the director Mr. John Changalucha for logistic support during fieldwork. I would also like to thank the field team of the parasitic diseases research programme, Mwanza Medical Research Centre for their active support during field data collection. Special thanks go to Drs. Godfrey Kaatano and Jabay E. Magesse for clinical and ultrasound examination of all children who participated in the study. The support provided by NIMR-Mwanza Centre data management unit particularly Mr. Coleman Kishamawe for database preparation, data entry and cleaning and assistance during data analysis is highly appreciated.

Apart from the field team in Tanzania, I would like to convey special thanks to the management and staff of the DBL-Centre for Health Research and Development, Faculty of Life Sciences (LIFE) of the University of Copenhagen for facilitating the part of the study which was done in Denmark and for training in various aspects related to the project. My special thanks particularly go to Ms Susanne Kronborg and Mrs Bente Larsen Jensen for the excellent training and guidance in ELISA techniques and for their assistance in the laboratory analysis for *P. falciparum* specific antibodies. Their dedicated laboratory work and moral support were an invaluable and indispensable aspect of the completion of this thesis work. I would like to thank Dr. Henry Madsen for the training in data management and analysis, particularly for introducing me to the use of STATA software for data analysis. I acknowledge the logistic support of DANIDA fellowship centre (DFC) during the writing phase of the thesis which took place in Denmark. Without the support of committed DFC staff, my stay in Denmark and hence the writing phase of the PhD thesis would not have been smooth and comfortable.

An equally important aspect to the successful completion of this study was the friendly cooperation of village leaders, teachers and parents of all villages and schools which participated in this study. Their cooperation was an important ingredient for the successful implementation of the data collection phase of this study. I would also like to acknowledge the support rendered to the project by the office of the district medical officer (DMO) and the office of the district education officer (DEO) for Magu district. In this regard, I would like to acknowledge the cooperation of Mr. Kaisaria Mambeya, district health officer and Ms. Veronica Justine, district education officer in-charge of school health programmes. Their support during fieldwork and their expert contributions during training workshops and dissemination meetings are acknowledged. Last but not least, I would like to register thanks to my wife Esther Innocent and our three children Noel, Frank and Emmanoela for their moral support and their patience during the entire period of the study. My wife Esther is thanked for her patience and for taking care of the family while I was away. This study was made possible through the financial support of the DBL-Centre for Heath Research and Development, Faculty of Life Sciences, University of Copenhagen and Danish Development Assistance (DANIDA), Denmark.

English Summary

The research work described in this thesis focuses on malaria and helminths (schistosome and soil transmitted helminths) co-infections in humans. Malaria, schistosomiasis and soil transmitted helminth infections (STH) are the most important parasitic infections in Sub-Saharan Africa (SSA), contributing to the biggest share of clinical disease burden. The higher prevalence of these infections and their overlap in geographical distribution in SSA results in higher rates of co-infections in humans that may affect disease progression, severity and outcome. The overall objective of the current study was to contribute to the knowledge on malaria and helminth (schistosomes and hookworm) co-infections in school and pre-school children with the ultimate goal of providing evidence based information that will support informed policy making on integrated parasitic diseases control programmes among school and pre-school children in Mwanza, Tanzania and elsewhere.

Chapter 1 provides background information on the study focusing on epidemiology and public health importance of malaria, schistosomiasis and STH infections in Tanzania and globally. A description of disease biology, transmission and clinical consequences of infections, rationale and objectives of the study is provided. An overview of the geographical overlap of malaria and helminth infections and the occurrence of co-infections in humans is provided. A comprehensive review of the literature on malaria and helminth co-infections in humans focusing on clinical consequences of co-infections, population groups at the highest risk and immunomodulation is provided. This chapter also explores the opportunities for integrated control of malaria, schistosomiasis and soil-transmitted helminth infections in at risk populations and age groups using available control tools such as cheap, safe and efficacious antihelmintic and insecticide treated nets (ITNs).

Chapter 2 presents an overview of the methodology and study design used. The study was implemented in Magu district, Mwanza region, Tanzania, from October 2006 to November 2008. The study was a prospective randomized controlled anthelmintic intervention trial with a baseline survey and two follow up surveys at 12 months and 24 months after baseline. The baseline survey which was conducted between October and November 2006 involved 1615 school and pre-school children from 6 selected primary schools namely Mwamayombo, Nyashimo, Bulima, Milambi, Ihale and Ijitu. The two years follow up surveys (1st follow up survey, October to November 2007 and 2nd follow up survey October to November 2008) involved a cohort of 765 children (who were initially infected with either *S. mansoni* or *S. haematobium* or both) selected from the 1615 children who participated the baseline survey. The 765 children were randomized into either an intervention group (394 children) or a control group (371 children). The intervention group was treated with praziquantel 40mg/kg and albendazole 400mg four times a year at three months interval while the control group was treated with praziquantel 40mg/kg and albendazole 400mg once a year. The chapter also provides information about the scientific approaches used to select the schools and the children included in the study, collection of stool, urine and blood samples in the field, clinical and ultrasound examination of all children, monitoring of clinical malaria attacks as well as laboratory techniques used to examine stool, urine and blood samples. In addition, the chapter gives an account of major outcome variables of the study and the ethical issues which were taken into considerations during implementation of the study.

Chapter 3 gives an account of the epidemiology of malaria and helminth co-infections in school and preschool children in Magu district. This chapter is based on a baseline survey that was implemented between October to November 2006 involving 1615 school and pre-school children.

The prevalence and infection intensities of malaria, *S. mansoni, S. haematobium* and hookworms for different schools, sex and age groups is given. In addition, the prevalence of multiple parasite infections involving *P. falciparum*, *S. mansoni, S. haematobium* and hookworms is also given. Chapter 3 also provides an account on haemoglobin concentrations and the prevalence of anaemia in the studied population. Associations between single and multiple parasitic infections as well as between parasitic infections and low haemoglobin levels and anaemia are also presented. Both malaria, *S. mansoni, S. haematobium* and hookworms were prevalent in the study area whereby 1079 (69.8%) out of 1546 children who were included in the data analysis were infected with at least one parasite. Polyparasitism was common in school and pre-school children in Magu district. Four hundred and thirty children (39.9%) had multiple parasite infections and 276 children (60%) of all children infected with *P. falciparum* were concurrently infected with at least one helminth specie. The most important parasite combinations involved *S. mansoni, P. falciparum and S. haematobium*. The prevalence of malaria parasitaemia was significantly associated with hookworm infection. Malaria parasite density decreased with increasing infection intensity of *S. mansoni* and *S. haematobium*. Likewise, malaria parasite densities decreased significantly with increasing number of co-infecting helminth species. Overall the prevalence of anaemia was 34.4% with the highest prevalence observed in children co-infected with *P. falciparum*, *S. mansoni* and *S. haematobium*. Anaemia was significantly more prevalent in children with multiple parasite infections (two parasites or more) compared to children infected with single or no parasite infection. Likewise, *P. falciparum* and *S. haematobium* infections were significant predictors of anaemia. Findings of this study suggest that polyparasitism is common in school and pre-school children in Magu district with the most important parasite combinations involving *S. mansoni, P. falciparum* and *S. haematobium*.

Chapter 4 focuses on assessment of *S. mansoni* and *S. haematobium* related morbidity using ultrasound and examines the relationship between morbidity and infection status. The chapter also gives a brief description of detection of organ enlargement (spleen, liver or both) by clinical examination using physical palpation. This results presented in this chapter are based on the baseline survey that was implemented between October to November 2006 and data from 1546 school and pre-school children from 6 schools/villages aged 3 – 13 years were included in the analysis. The prevalence and severity of liver, spleen and urinary tract pathology and its association with both the prevalence and infection intensity of *S. mansoni* and *S. haematobium* (as appropriate) is given. The chapter also discusses the effect of *P. falciparum* as a single infection or in combination with other infections on organ pathology. *S. mansoni* and *S. haematobium* related pathology was prevalent in the study area and correlated well with both prevalence and infection intensity of *S. mansoni*, *S. haematobium* and malaria infection. The prevalence of *S. mansoni* and *S. haematobium* related pathology differed significantly among schools reflecting variation in transmission and duration of *S. mansoni* and *S. haematobium* infection from one location to another. Further, hepatosplenic disease (hepatosplenomegaly) was common in children as young as 5 years confirming previous studies in the area which found higher prevalence of *S. mansoni* related pathology particularly in adults.

Chapter 5 looks at the impact of two anthelmintic treatment approaches on malaria infection, anaemia and on schistosome and STH infections in school and preschool children by comparing two randomised treatment groups: an intervention group of 394 children treated with praziquantel 40mg/kg and albendazole 400mg four times a year at three months interval and a control group of 371 children treated with praziquantel 40mg/kg and albendazole 400mg once a year. The two groups were followed up for two years at 12 months interval. The outcome of interest was changes in prevalence and infection intensity of *P. falciparum* infection, changes in prevalence of anaemia and haemoglobin levels and changes in prevalence and infection intensity of *S. mansoni, S. haematobium* and hookworm. In addition, frequency of malaria attacks in both the intervention and

control groups and changes in prevalence of different morbidity indicators were assessed. Overall, the quarterly antihelmintic treatment approach did not have an impact on malaria infection (prevalence, malaria parasite density and frequency of malaria attacks) or on prevalence of anaemia. However, the quarterly anthelmintic treatment approach significantly reduced prevalence and intensity of *S. mansoni, S. haematobium* and hookworms compared to the standard single dose annual treatment. In addition, the quarterly anthelmintic treatment approach had a significant effect on prevalence of multiple parasite infections. There were reductions in the prevalence of periportal fibrosis, splenomegaly, hepatosplenomegaly and enlarged portal vein diameter over the two years follow up period but without significant differences between groups. Haemoglobin concentrations increased significantly in both the intervention and control groups which in turn resulted into significant reduction in prevalence of anaemia in both groups. These results indicate that an antihelminic intervention to control helminth infections may not have a direct impact on malaria parasitaemia, infection density or clinical malaria. However the intervention has a significant impact on overall prevalence of single and multiple infections. The chapter highlights the importance of an integrated approach for the control of parasitic infections in school and pre-school children.

Chapter 6 reports on the impact of helminth infections (schistosome and STH) and the impact of an anthelmintic treatment intervention on *P. falciparum* specific immune responses among school and preschool children with emphasis on seroprevalence and levels of immunoglobulin G3 (IgG3) against *P. falciparum* schizont antigen (PfSE-IgG3). A total of 2822 serum samples were prepared from blood collected from 1572 children (baseline survey), 658 children (first follow up survey) and 592 children (second follow up survey). The immune response against *P. falciparum* infection was measured by determination of the level of IgG3 against *P. falciparum* schizont antigen (PfSE-IgG3) using the Enzyme Linked Immunosorbent Assay (ELISA) method. Out of 1505 children with complete baseline information, 1247 (82.9%) were seropositive for PfSE-IgG3. The seroprevalence and geometric mean levels of PfSE-IgG3 increased with age and differed significantly among schools. The seroprevalence of PfSE-IgG3 was significantly higher in children infected with *P. falciparum*, *S. haematobium* and hookworm compared to children without any infection. Children with co-infections of *P. falciparum* and *S. haematobium* had significantly higher levels of PfSE-IgG3 responses compared to uninfected children or children with *P. falciparum* infection only. In multivariate linear regression analysis, age group, *P. falciparum, S. haematobium* and hookworm infections were significant predictors of PfSE-IgG3 levels after adjusting for sex. In a multivariate logistic regression analysis PfSE-IgG3 was an important predictor of both splenomegaly and hepatosplenomegaly. The antihelmintic treatment intervention resulted into significant increase in PfSe-IgG3 levels particularly in older children (9-13 years) probably through mechanisms involving altered immune responses to schistosome antigens following treatment. However, the frequency of treatment (4 times a year vs. once a year) did not have an impact on both seroprevalence and levels of PfSE-IgG3. This chapter has demonstrated the importance of helminth co-infections particularly *S. haematobium* and hookworm and the influence of antihelmintic treatment on anti-*P. falciparum* immune responses. However, it is not clear if this is associated with improved protection against *P. falciparum* infection and disease.

Chapter 7 provides a discussion of the major findings of the study while highlighting some important contributions to the understanding of the epidemiology and control of parasitic infections and co-infections in humans. Emphasis is provided on the public health importance of parasitic co-infections in school and pre-school children and on the importance of an integrated approach to control these infections. The chapter also proposes areas for further research.

Dansk resume

PhD afhandlingen omhandler forskellige aspekter af samtidig infektion med malaria og helminter; her schistosomer og indvoldsorm. Malaria, schistosomiasis og infektion med indvoldsorm er de hyppigst forekommende parasitinfektioner i Afrika syd for Sahara, hvor de bidrager til en meget væsentlig del af sygdomsbyrden. En høj prævalens af alle tre typer af infektion samtidig med et overlap i den geografiske udbredelse medfører en høj forekomst af saminfektion med to eller flere af parasitterne hvilket kan påvirke infektionernes forløb og sværhedsgrad. Det overordnede formål med undersøgelsen var at bidrage til viden omkring betydningen af samtidig infektion med malaria og helminter blandt børn i førskole- og skolealderen med det overordnede mål at fremskaffe evidensbaseret information til støtte for forbedrede integrerede parasit kontrol programmer blandt førskole- og skolebørn i Tanzania så vel som andre steder.

Kapitel 1 præsenterer baggrundsinformation for studiet med fokus på epidemiologi og den samfundsmedicinske betydning af malaria, schistosomiasis og indvoldsorm såvel i Tanzania som globalt. Infektionernes sygdomsbiologi, transmission og kliniske betydning, rationalet bag studiet og studiets formål og delformål beskrives. Ydermere berøres emner som det geografiske overlap mellem malaria og helmintinfektionerne og forekomsten af samtidige infektioner i mennesker. Kapitel 1 giver en grundig sammenfatning af litteraturen omkring samtidig infektion med malaria og helminter i mennesker med fokus på det kliniske billede, højrisiko populationer og potentiel immunmodulerende effekt af infektionerne. Desuden diskuteres mulighederne for integreret kontrol af malaria, schistosomiasis og intestinale helmintinfektioner blandt højrisikogrupper og i forskellige aldersgrupper med brug af billige, sikre og effektive ormemidler og insekticidbehandlede myggenet.

Kapitel 2 gennemgår designet af studiet og de metoder der er anvendt for indsamling af data. Studiet blev udført i Magu distrikt, Mwanza region, Tanzania fra oktober 2006 til november 2008. Det var designet som et prospektivt randomiseret kontrolleret interventionsstudie startende med en tværsnitsundersøgelse efterfulgt af to opfølgningsstudier 12 og 24 måneder efter studiets start. Det initiale tværsnitsstudie blev udført mellem oktober og november 2006 og involverede 1615 skole- og forskolebørn fra følgende seks udvalgte skoler; Mwamayombo, Nyashimo, Bulima, Milambi, Ihale and Ijitu. Et- og toårs opfølgningsundersøgelserne (oktober til november 2007 og oktober til november 2008) involverede en kohorte af 765 børn (som ved studiets start var inficeret enten med *Schistosoma mansoni, S. haematobium* eller begge dele) udvalgt blandt de 1615 børn, som deltog i den initiale undersøgelse. Kohorten på 765 børn blev randomiseret til en interventionsgruppe (394 børn) eller en kontrolgruppe (371 børn). Interventionsgruppen blev behandlet med praziquantel (40 mg/kg) og albendazol (400 mg) fire gange om året med tre måneders interval mens kontrolgruppen blev behandlet med praziquantel (40 mg/kg) og albendazol (400 mg) en gang årligt. Kapitel 2 beskriver også de metoder der er anvendt i studiet for eksempel udvælgelsen af skolerne, indsamling af fæces, urin og blod i felten, klinisk og ultralydundersøgelse af børnene, monitorering af kliniske malariaepisoder så vel som laboratorieteknikker anvendt til undersøgelse af fæces, urin og blodprøver. Desuden beskrives de vigtigste udfaldsparametre og etiske aspekter som blev taget i betragtning ved planlægning og udførelse af studiet.

Kapitel 3 er baseret på den initiale tværsnitsundersøgelse i oktober til november 2006 og beskriver de epidemiologiske fund vedrørende malaria og helmintinfektioner blandt de skole og førskolebørn der indgik i studiet. Prævalens og infektionsintensitet af malaria, *S. mansoni*, *S. haematobium* og hageorm er angivet for de forskellige skoler samt opdelt efter køn og forskellige aldersgrupper. Desuden er prævalensen af multiple parasitinfektioner involverende *P. falciparum*, *S. mansoni*, *S. haematobium* og hageorm angivet. Kapitlet præsenterer også data vedrørende koncentrationen af

hæmoglobin og prævalensen af anæmi i studiepopulationen samt associationer mellem enkelte og multiple parasitinfektioner samt mellem parasitinfektioner og lav hæmoglobin og prævalensen af anæmi. Prævalensen af malaria, schistosomiasis og hageormsinfektion var høj og 1079 (69,8%) ud af de 1546 børn der indgik i dataanalysen var inficeret med mindst en af disse parasitter. Polyparasitisme var hyppigt forekommende; 430 (39,9%) af børnene havde multiple parasitinfektioner og 276 (60%) af de børn der var inficerede med *P. falciparum* var samtidig inficeret med mindst en helmint type. Prævalensen af malaria parasitæmi var signifikant associeret med infektion med hageorm. Densiteten af malaria parasitæmi faldt med stigende infektionsintensitet af *S. mansoni* og *S. haematobium.* Tilsvarende faldt malariaparasit densiteten signifikant med stigende antal af forskellige helmintinfektioner. Prævalensen af anæmi var 34,4% og den højeste prævalens blev observeret blandt børn som var inficeret med både *P. falciparum, S. mansoni* og *S. haematobium.* Anæmi var signifikant hyppigere blandt børn med multiple parasitinfektioner (to eller mere) end blandt børn med en enkelt eller ingen parasitinfektioner. Studiet viser at polyparasitisme er hyppigt forekommende blandt skole- og førskolebørn I Magu Distrikt og de hyppigst forekommende kombinationer af infektioner involverer *S. mansoni, P. falciparum* og *S. haematobium*.

Kapitel 4 fokuserer på bestemmelse af *S. mansoni* og *S. haematobium* relateret morbiditet ved hjælp af ultralydundersøgelse og undersøger sammenhængen mellem morbiditet og infektionsstatus. Kapitlet giver desuden en beskrivelse af organforstørrelse (hepato- splenomegali) bedømt ved palpation. Resultaterne i dette kapitel er baseret på den initiale tværsnitsundersøgelse foretaget oktober til november 2006 og data fra 1546 skole- og førskolebørn fra 6 skoler/landsbyer indgik i undersøgelsen. Forekomsten og graden af patologiske forandringer i lever, milt og urinveje og associationen med prævalens og intensitet af *S. mansoni* og *S. haematobium* præsenteres. Desuden diskuteres effekten af *P. falciparum* infektion, enten som enkeltinfektion eller i kombination med helmintinfektion, på forekomsten og graden af organrelateret patologi. Patologiske forandringer relateret til *S. mansoni* og *S. haematobium* var hyppigt forekommende og korrelerede med både prævalens og intensitet af *S. mansoni, S. haematobium* og malaria infektion. Der var signifikant forskel i prævalensen af *S. mansoni* og *S. haematobium* relateret patologi mellem de forskellige skoler; et forhold der reflekterede forskelle i transmission i *S. mansoni* og *S. haematobium* fra lokalitet til lokalitet. Ydermere var organomegali (hepatosplenomegali) hyppigt blandt børnene og blev observeret blandt børn helt ned til 5 års alderen hvilket understøtter fund fra tidligere studier i området som har beskrevet en høj forekomst af *S. mansoni* relateret morbiditet specielt blandt voksne.

I kapitel 5 undersøges to forskellige behandlingsstrategiers effekt på malaria, anæmi, schistosomiasis og infektion med indvoldsorm blandt førskole- og skolebørn. De to randomiserede grupper der sammenlignes er: En interventionsgruppegruppe bestående af 394 børn der behandles med praziquantel (40mg/kg) og albendazol (400 mg) fire gange årligt med tre måneders interval og en kontrolgruppe på 371 børn, der behandles med praziquantel (40mg/kg) og albendazol (400 mg) en gang årligt. De to grupper blev fulgt i to år med 12 måneders interval. Følgende parametre blev undersøgt: Ændringer i prævalens og intensitet af *P. falciparum* infektion, ændring i forekomst af anæmi og hæmoglobin koncentration samt ændringer i prævalens og intensitet af *S. mansoni, S. haematobium* og hageorm. Desuden blev hyppigheden af malariaepisoder undersøgt i interventions- såvel som i kontrolgruppen og indikatorer for morbiditet blev ligeledes målt i begge grupper. Generelt fandt man ingen øget effekt på malaria infektionsparametre (prævalens, malariaparasit densitet, hyppighed af malariaepisoder) eller forekomsten af anæmi af at behandle fire gange årligt. Derimod blev prævalens og intensitet af *S. mansoni, S. haematobium* og hageorm signifikant reduceret i interventionsgruppen sammenlignet med kontrolgruppen. Desuden sås en signifikant reduktion i prævalensen af multiple parasitinfektioner i interventionsgruppen sammenlignet med

kontrolgruppen. Der var reduktion af periportal fibrose, hepatosplenomegali, forstørret portalvenediameter og patologiske forandringer i urinvejene ved to års opfølgningsundersøgelsen men uden signifikant forskel mellem interventions- og kontrolgruppen. Koncentrationen af hæmoglobin øgedes signifikant i begge grupper og dette resulterede i en tilsvarende reduktion i prævalensen af anæmi. Kapitlet understreger vigtigheden af integreret kontrol af parasitinfektioner blandt førskole- og skolebørn i endemiske områder.

Kapitel 6 omhandler effekten af helmintinfektioner (schistosomiasis og indvoldsorm) og anthelmintika behandling på *P. falciparum* specifikke antistoffer; i dette tilfælde seroprævalens og niveau af IgG3 rettet mod *P. falciparum* schizont antigen (PfSE-IgG3). I alt indgik 2822 blodprøver i studiet indsamlede på tre tidspunkter nemlig ved studiets start (1572 prøver) og ved henholdsvis et- og toårs opfølgningen (henholdsvis 658 og 592 prøver). Antistoffer rettet mod *P. falciparum* (PfSE-IgG3) blev målt i et Enzyme Linked Immunosorbent Assay (ELISA). Ud af 1505 børn med komplet baseline information fandtes 1242 (82,9%) at være seropositive. . Både seroprævalensen og niveauet af PfSE-IgG3 steg med alderen og der sås signifikante forskelle skolerne imellem. Seroprævalensen af PfSE-IgG3 var signifikant højere blandt børn med *P. falciparum, S. haematobium* og hageorm infektion sammenlignet med børn uden infektion. Børn der var dobbelt-inficerede med *P. falciparum* og *S. haematobium* og de der var dobbelt-inficerede med *P. falciparum* og hageorm havde signifikant højere PfSE-IgG3 niveauer end uinficerede børn eller børn som kun var inficerede med *P. falciparum.* I multivariat lineær regressionsanalyse var aldersgruppe og infektion med *P. falciparum, S. haematobium* og hageorm signifikante prædiktorer af PfSE-IgG3 niveauet efter justering for køn. I multivariat logistic regressionsanalyse var PfSE-IgG3 en signifikant prædiktor for både splenomegali og hepatosplenomegali. Behandling med praziquantel og albendazol resulterede i en signifikant stigning i PfSE-IgG3 niveauet specielt blandt de ældre børn (10-13 år) muligvis gennem en mekanisme der involverer et ændret immunrespons til schistosomantigener som resultat af behandlingen. Behandling fire gange årligt (interventionsgruppen) havde dog ingen yderligere effekt hverken på seroprævalensen eller niveauet af PfSE-IgG3. Dette kapitel har vist at samtidig infektion med helminter, specielt *S. haematobium* og hageorm og indflydelsen af ormebehandling på anti-*P. falciparum* antistof responset, omend det ikke kan belyses ud fra disse resultater om dette er associeret med øget beskyttelse mod *P. falciparum* infektion eller morbiditet.

I kapitel 7 diskuteres de væsentligste fund fra studiet idet der fokuseres på de resultater, der kan bidrage til forståelsen af epidemiologi og kontrol af multiple parasitinfektioner hos mennesker. Der lægges vægt på den folkesundhedsmæssige betydning af samtidige parasitinfektioner bland førskole- og skolebørn og på vigtigheden af en integreret tilgang til kontrol af disse infektioner. Til slut præsenteres forslag til fremtidige forskningsområder indenfor feltet.

List of Tables

List of Figures

List of Abbreviations

ACT	Artemisinin based Combination Therapy
ALB	Albendazole
ANOVA	Analysis of variance
APOC	African Programme for Onchorcerciasis Control
CI	Confidence Interval
^0C	Degrees Celsius
ELISA	Enzyme Linked Immunosorbent Assay
Epg	Eggs per gram of faeces
Hb	Haemoglobin
χ^2	Chi-square
IFN	Interferon
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IL	Interleukin
IMCI	Integrated Management of Childhood Illnesses
IP	Image pattern
IPT	Intermittent Preventive Treatment
IRS	Indoor Residual Spraying
ITN	Insecticide Treated Nets
MAL	Mid axillary line
MCL	Mid clavicular line
MDA	Mass drug administration
MoHSW	Ministry of Health and Social Welfare
MPS	Malaria parasites
MRCC	Medical Research Coordination Committee
MSL	Mid sternal line
MSP-1	Merozoite surface protein 1
NBS	National Bureau of Statistics
NIMR	National Institute for Medical Research
OD	Optical density
OPD	Orthophenylene Diamine Dihydrochloride
PfSE	*P. falciparum* schizont antigen
PfSE-IgG3	Immunoglobulin G3 against *P. falciparum* schizont antigen
PPF	Periportal fibrosis
PSL	Para sternal line
PVD	Portal vein diameter
PZQ	Praziquantel
SCI	Schistosomiasis Control Initiative
SEA	Soluble egg antigen
STH	Soil-Transmitted Helminth Infections
SSA	Sub-Saharan Africa
TNF	Tumor necrosis factor
Th1	T-helper cells type I
Th2	T-helper cells type II
µl	Microlitre
WBC	White blood cells
WHO	World Health Organization

Chapter 1: General introduction

1.1. Malaria, schistosomiasis and soil-transmitted helminth infections

1.1.1. Epidemiology and public health importance

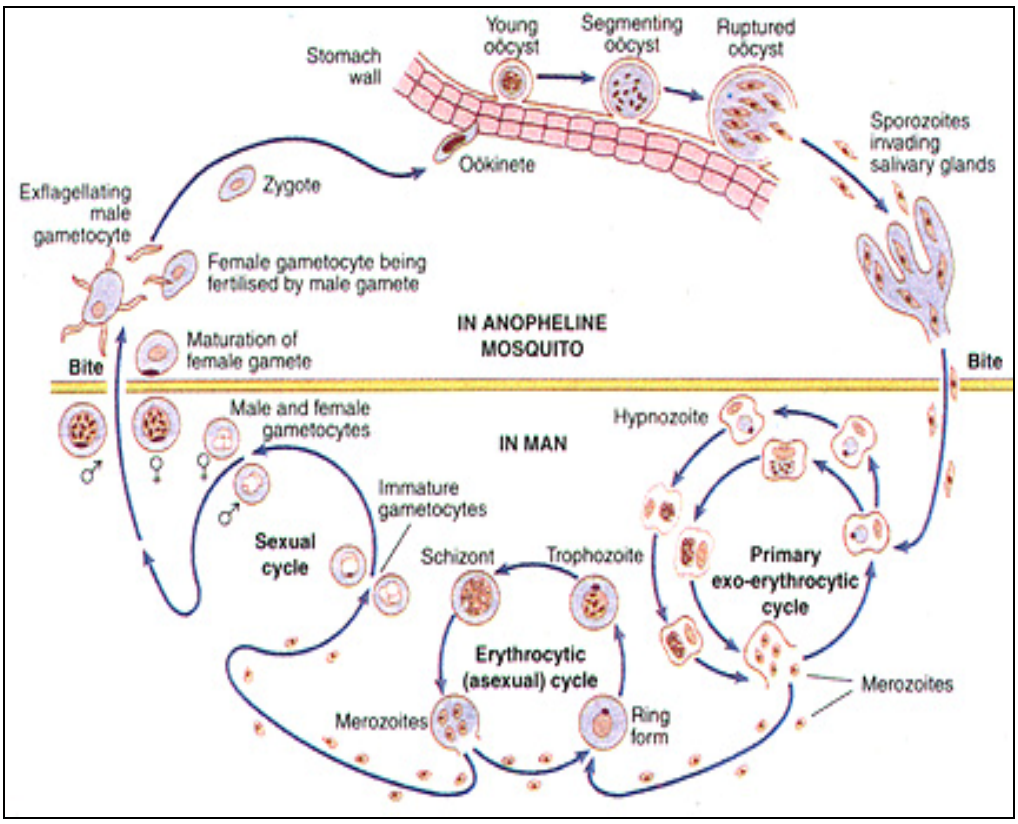
Malaria, schistosomiasis and soil transmitted helminth infections (STH) are the most important parasitic infections in Sub-Saharan Africa (SSA), contributing to the biggest share of clinical disease burden (WHO, 2002). In SSA, malaria is caused mainly by *P. falciparum*. Overall, it is estimated that there are about 247 million malaria cases (189 – 327 million) and 881,000 deaths (610,000 – 1,212,000) worldwide of which 90% occur in SSA (WHO, 2008).

In SSA, schistosomiasis is caused by *Schistosoma mansoni* and *Schistosoma haematobium.* More than 80% of global schistosome infections occur in SSA (Chitsulo, 2000). Soil transmitted helminth (STH) infections are also common in the area and are caused by the hookworms *Necator americanus* and *Ancylostoma duodenale*, *Ascaris lumbricoides* and *Trichuris trichiura*. Schistosomiasis and STH are particularly widely distributed in poor populations of less developed countries and thus closely associated with poverty. The burden of disease caused by infections with schistosomiasis and STH is enormous. It is estimated that about 2 billion people are affected worldwide of whom 400 million suffer associated severe disease. In 1999 WHO estimated that schistosomiasis and STH infections represented more than 40% of the disease burden caused by all tropical diseases excluding malaria (WHO, 2006). There is evidence to suggest that the prevalence of schistosomiasis particularly of *S. mansoni* is increasing due to water resource development projects, population increase or displacement, human migration and competing priorities in the health sector (Chitsulo *et al*, 2000; Engels *et al*, 2002). An annual mortality rate due to Schistosomiasis in SSA is estimated to exceed 200,000. The estimated number of cases with bladder wall pathology, hydronephrosis and hepatosplenic disease is estimated at 18, 10 and 8.5 million, respectively (Van der werf *et al*, 2003). STH may cause up to 135,000 deaths a year but the major public health significance is related to chronic morbidity on health and nutrition status which compromise physical and intellectual development resulting to stunted growth and poor academic achievement in school children and poor pregnancy outcomes in pregnant mothers (WHO, 2002; WHO 2006).

1.1.2. Biology and transmission

Malaria is transmitted to humans through the bite of a wide variety of anopheline mosquitoes. There are four known species of plasmodium that cause malaria in humans namely *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. However, in Africa, more than 75% of severe disease and deaths are due to *P. falciparum* (White, 2009). The biology of malaria parasites involves a sexual phase (sporogony) in the vector female anopheles mosquito and an asexual phase (schizogony) in the human host. Sporozoites are inoculated into the human host by feeding female anopheles mosquitoes where they develop in liver parenchyma cells to form hepatic schizonts. The mature hepatic schizont ruptures and releases merozoites which invade red blood cells where they grow and multiply to form blood schizonts. Multiplying blood schizonts destroy red blood cells causing malaria disease. Some forms of the parasites (merozoites) develop into sexual forms called gametocytes which are picked by female anopheles mosquitoes during a blood meal and injected to another susceptible human host, where they start another cycle of development to form human infective sporozoites (White, 2009).

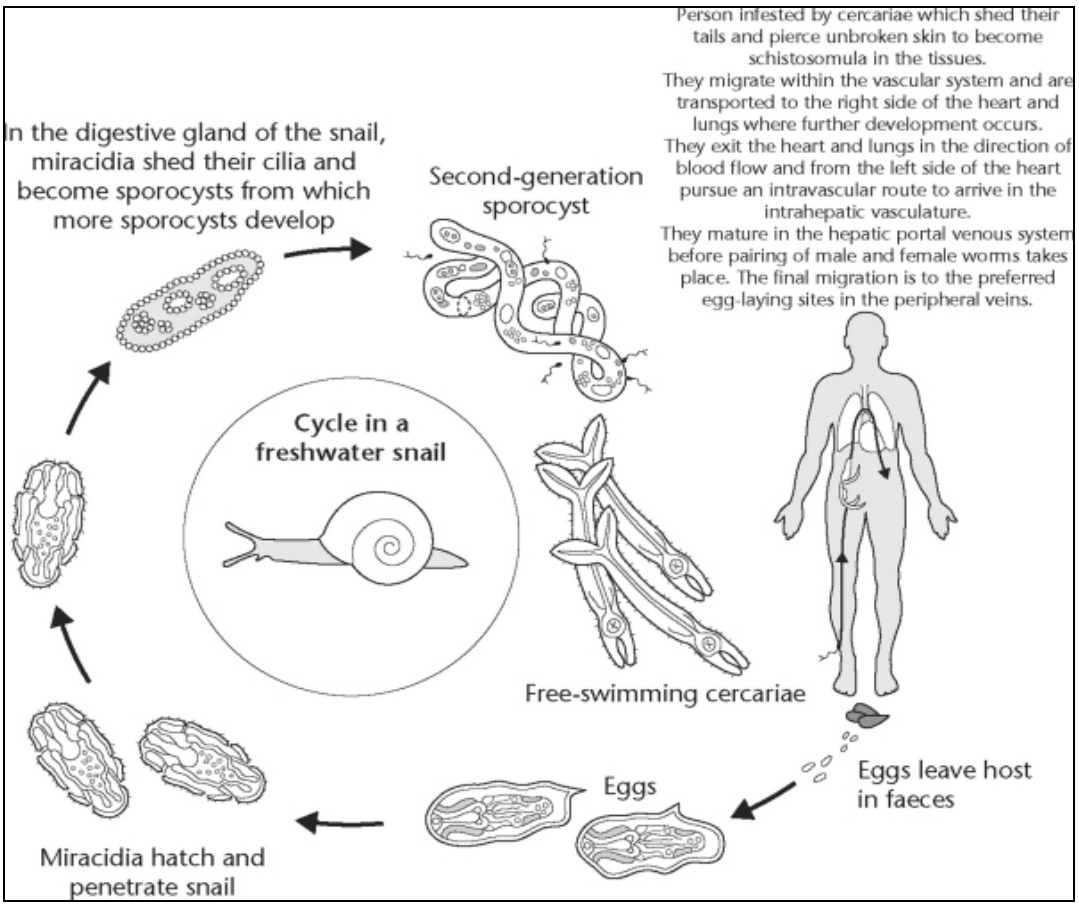
Figure 1.1 Life cycle of human malaria parasites



(Reproduced from: http://www.malariasite.com/malaria/LifeCycle.htm).

Human schistosomiasis is caused by infection with blood flukes of the genus *Schistosoma*. There are five major species of schistosomes namely *S. mansoni, S. haematobium, S. japonicum, S. mekongi and S. intercalatum* (Rolllingson and Southgate, 1987). The major species in Africa are *S. mansoni* that causes intestinal schistosomiasis and *S. haematobium* that causes urinary schistosomiasis. The distribution of schistosomiasis is linked to the distribution of the snail intermediate hosts. *S. mansoni* occurs in Africa and South America and is transmitted by *Biomphalaria spp. S. haematobium occurs* in Africa and the Middle East and is transmitted by *Bulinus spp. S. japonicum* occurs in South East Asia, China and the Philippines and is transmitted by *Oncomelania spp.* (Rolllingson and Southgate, 1987). Human schistosomes have a complex life cycle involving the human host and the intermediate host fresh water snail. The snail intermediate host sheds free swimming cercariae that penetrate the skin of human when in contact with water. After penetration of human skin, within 24 hours, the cercariae transform into schistosomula, which migrate through the veins and lymph vessels to the lungs and liver where they develop into male and female worms in the portal blood vessels. The female worm lies within the gynaecophoric canal of the male worm. After 4 - 6 weeks, mating takes place and the worms-pairs move to their final destinations. *S. mansoni* adults migrate to the portal veins draining the large intestines, *S. japonicum* migrate to the veins of the small intestines and *S. haematobium* migrate to the plexus of the urinary bladder. The period between penetration of cercariae to egg laying is called the prepatent period and is about 30 – 40 days for *S. mansoni* and 54 – 84 days for *S. haematobium* (Ukoli, 1984; Sturrock, 1987; Davis, 2009). Eggs produced by female worms penetrate the walls of blood vessels and are shed in urine (*S. haematobium* ova) or in faeces (*S. mansoni and S. japonicum* ova) thus completing the cycle. When laid egg reaches water, they hatch within a few minutes into another free swimming larva, the miracidium. The miracidia can stay in water for about 24 hours before encountering and penetration of a specific snail intermediate host where they develop into a sporocyst and then into a human infective cercariae (Davis, 2009).

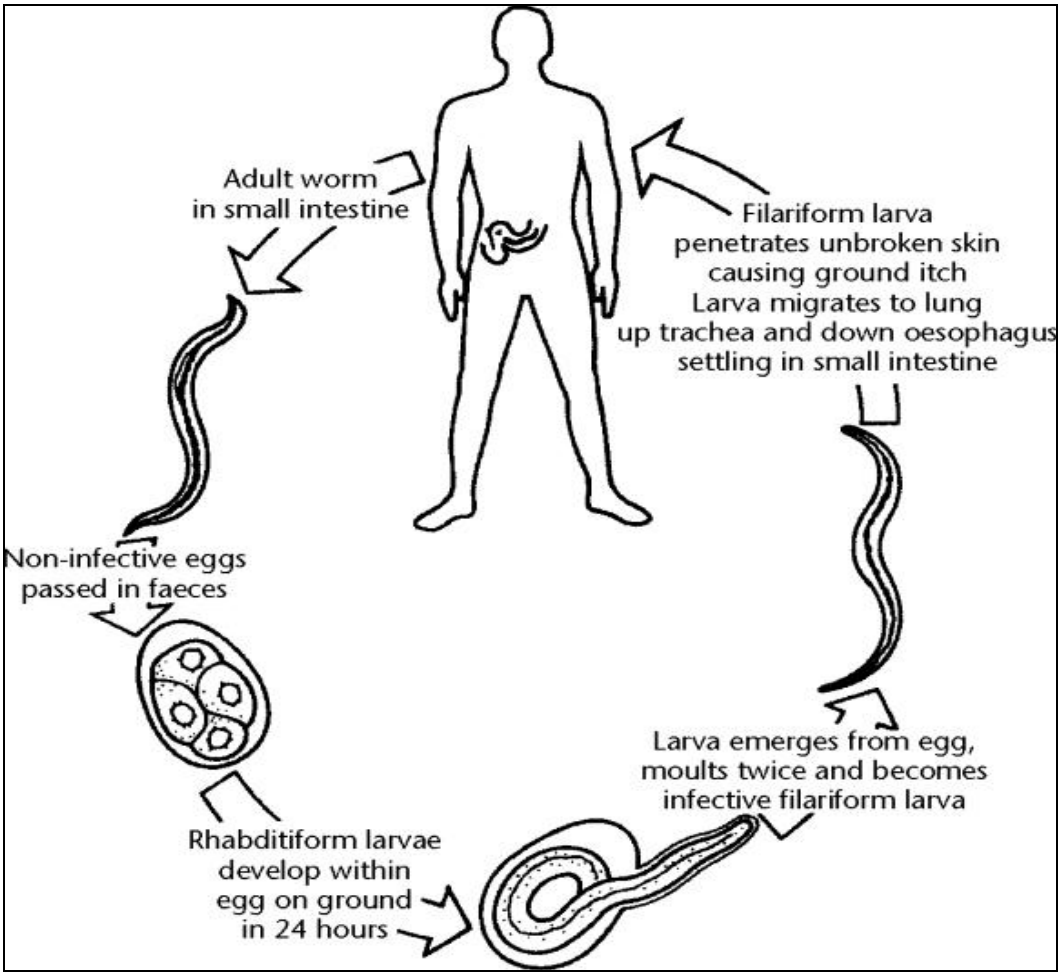
Figure 1.2 Life cycle of *S. mansoni*



(Reproduced after Davis, 2009)

The life cycles of the major STHs hookworm, *A. lumbricoides* and .*T. trichiura* have many features in common. The adult parasites inhabit the human intestinal tract. For hookworms, human acquire the infection when third stage larvae in soil penetrate their skin while for *A. lumbricoides* and *T. trichiura*, infection takes place through the oral route by contaminated food or water. In some settings, transmission can occur through the practice of eating soil or geophagia (Geissler *et al*, 2009). Larvae migrate through the vasculature and are carried to the muscles of the heart and the lungs. From the lung tissues, the larva ascend through the alveoli, bronchioles, bronchi and trachea from where they are coughed and swallowed into the intestinal tract. In the tissues of the intestines, they develop into adults and lay eggs. Except for *A. lumbricoides* which does not develop in soil, worm eggs shed into the soil through faeces hatch into first stage larvae which in turn hatch twice to form the infective third stage larvae upon contact with adequate moisture and temperature (Hotez *et al*, 2004; Brooker and Bundy, 2009). Unlike schistosomes, STH life cycle is direct and doesn't involve intermediate hosts. Eggs hatch in the soil and the infective third stage larvae develops in the soil (Ukoli, 1984).

Figure 1.3. Life cycle of hookworm



(Reproduced after Simon Brooker and Bundy, 2009)

Adult worms live in the human intestine and produce up to 30,000 eggs per day. Eggs are deposited into intestinal lumen from where they are discharged into soil through faeces. In the soil, with the right moisture and temperature, they hatch into free living rhabditiform first-stage larvae. The first stage larva molts twice to form an infective filariform third stage larva which is motile. Upon contact with host skin, the third stage larvae penetrates it, enters vasculature and starts migrations reaching the lungs, bronchioles, trachea and down to the oesophagus, stomach and small intestine. In the intestines, the larvae develop into adult worms, attaches themselves to intestinal mucosa and lays eggs.

1.1.3. Clinical consequences of malaria, schistosomiasis and STH infections

The clinical consequences of infection with malaria, schistosomes and STH are related to tissue damage, blood loss, anaemia and host immune responses. Malaria infection causes destruction of erythrocytes and the liberation of parasite and erythrocyte materials into host circulation. Further, *P. falciparum* infected erythrocytes sequester in the microcirculation of vital organs, interfering with microcirculatory flow and host tissue metabolism. The contents of destroyed red blood cells that are released into bloodstream stimulate release of tumor necrosis factor (TNF) and other cytokines. The released cytokines are responsible for the symptoms of the infection, particularly fever, chills, headache and malaise. Malaria may develop into severe malaria with hyperthermia, severe anaemia,

jaundice, cerebral malaria and enlargement of the liver and spleen. If not treated, malaria may cause impaired kidney, liver, heart, lungs and brain functions and death (White, 2009).

In schistosome infection, schistosome eggs cause tissue damage and blood loss in urine or faeces depending on species involved. Schistosome disease is caused by immune reaction of the host against deposited eggs. Schistosome eggs provoke host inflammatory response which is a manifestation of a delayed hypersensitivity reaction mediated through T-lymphocytes, macrophages and eosinophils (Davis, 2009). This reaction leads to granuloma formation in the genito-urinary organs in the case of urinary schistosomiasis, or intestines (colon) and liver in the case of intestinal schistosomiasis (Davis, 2009). Chronic infection with *S. mansoni* result into periportal fibrosis, hepatosplenic involvement with liver and spleen enlargement, portal hypertension and oesophageal varices (Warren, 1987; Davis, 2009). In the case of *S. haematobium* infection, late stage sequelae include hydronephrosis, bladder wall irregularity and calcification and predisposition to bladder cancer (Warren, 1987).

Most STH infections do not result into clinical disease. This is because clinical disease is associated with intensity of infection and only in individuals with heavy worm burden, infection results into overt disease characterised by anaemia, impaired growth and cognition, as well as impaired food digestion and absorption (Brooker and Bundy, 2009). The major pathogenic injury in STH infections is related to tissue damage, blood and protein loss. In addition, migration of STH larvae causes tissue damage and haemorrhages in affected organs (Ukoli, 1984; Hotez *et al*, 2004). In moderate to heavy hookworm infection there is iron deficiency anaemia which results from chronic blood loss, depletion of iron stores and impaired dietary iron intake. In addition, there is loss of protein and albumin which in turn results into hypoproteinaemia, hypoalbuminaemia and generalised oedema. Clinically, hookworm disease is characterised by paleness particularly of mucous membranes (anaemia), weakness, swelling of feet and ankle and low haemoglobin levels. There is also pulmonary and abdominal involvement such as coughing, asthmatic wheezing and abdominal pains (Hotez *et al*, 2004; Stoltzfus *et al*, 1997; WHO, 2002, Brooker and Bundy, 2009). Studies conducted in Tanzania show a correlation between the burden of hookworm infection and depletion of iron stores (Stoltzfus *et al*, 1997). In women of reproductive age, hookworm infection contributes to depletion of iron stores which may causes severe anaemia in women with heavy infections, low birth weight of their infants and neonatal prematurity (Bundy *et al*, 1995; Christian *et al*, 2004). In *A. lumbricoides* infection, pathology and disease are caused by adult worms and migration of larva and is related to the intensity of infection. Migrating larvae cause damage of lung tissues, haemorrhages and eosinophilic inflammatory responses. In the liver, migrating larvae cause small areas of necrosis with eosinophil infiltration. Lesions similar to those observed in visceral leishmaniasis can also be observed in the eyes, skin and brain. Heavy burden of *A. lumbricoides* adult worms cause intestinal colic, volvulus, intestinal obstruction or intussusception. Wandering *A. lumbricoides* worms also cause mechanical obstruction, intestinal perforation and acute appendicitis. They can also cause obstructive jaundice, liver abscesses, pancreatic necrosis and oesophageal perforation. Granulomatous lesions are found around dead adult worms and eggs in the liver and bowels. Clinically, *A. lumbricoides* infection is characterised by acute abdominal pain, cough, pneumonitis and asthma. The liver is enlarged and tender. *A. lumbricoides* infection can cause malabsorption of macro and micronutrients resulting into nutritional deficiency and growth retardation (Brooker and Bundy, 2009). Infection due to *T. trichiura* causes damage to intestinal mucosa resulting into haemorrhages, mucopurulent stools, dysentery and rectal prolapse. There is mucosal damage which facilitates invasion by other parasites such as entamoeba histolytica, shigellosis and *Campylobacter jejuni*. Clinically, *T. trichiura* infection is characterised by severe dysentery with blood and mucus and prolapse of the rectum. There is hypoproteinaemia, severe anaemia and growth retardation. Overall, STH infections in children are associated with impaired physical and mental development and severe under nutrition (Stephenson *et al*, 2000, WHO, 2002; Hotez *et al*, 2004).

1.2. Malaria and helminth co-infections

1.2.1. Geographical overlap in distribution and occurrence of co-infections in humans

In SSA, a number of helminth species share the same geographical distribution with *P. falciparum* (De Silva *et al*, 2003; Utzinger *et al*, 2004). The higher prevalence of these parasitic infections in this region and their overlap in geographical distribution result in high rates of co-infections in humans (De Silva *et al*, 2003; Van der Werf *et al*, 2003; Snow *et al*, 2005; Brooker *et al*, 2006; Brooker *et al*, 2007). Previous studies have demonstrated that about a quarter of African school children are concurrently infected with *P. falciparum* and hookworms (Brooker *et al*, 2006). The overlapping distribution of various parasites is an important factor in considering the risk of co-infections and co-morbidity in the population. The risk of exposure to co-infections and co-morbidity depend on the level of transmission of the parasite species in the area, the age of exposed individuals and or acquisition of immunity (Snow *et al*, 1997; Brooker *et al*, 2007). The schistosome species *S. mansoni*, *S. haematobium* and the STHs *A. lumbricoides* and *T. trichiura* are common in children aged 5-14 years. Hookworm infections also occur in this age group but peaks in early adulthood (Bundy, 1998). Severe malaria is uncommon in this age group but clinical malaria episodes and asymptomatic infections are common (Kimbi *et al*, 2005; Clarke *et al*, 2004; Mwangi *et al*, 2006). Malaria and helminth infections are also common in pregnant women (Shulman *et al*, 1999). It is evident that the occurrence of malaria and helminth co-infections and the possible clinical consequences are more important in school aged children and pregnant women than in other population groups (Mwangi *et al*, 2006).

1.2.2. Helminth co-infections and immunomodulation

Acquired immunity against *P. falciparum* infection can be achieved either through cell mediated immunity or humoral (antibody) mediated immunity (Marsh, 2002). Cell mediated immunity depends on T-lymphocytes which act through a variety of mechanisms such as direct demage of invading agents through phagocytosis or antiboody dependent cell mediated cytotoxicity (Crompton *et al* 2010; Bouharoun-Tayoun *et al*, 1990). Humoral mediated immunity depends on B-lymphocytes which with the assistance of specialised cells called T-helper (Th) cells produce specialised effector molecules called immunoglobulins (antibodies) (Marsh, 2002). The key components of antibodies against *P. falciparum* infection are immunoglobulin G isotypes mainly IgG1 and IgG3 which is an indication of a T-helper cells type 1 (Th1) mediated immune response (Cohen *et al*, 1961; Sabchareon *et al*, 1991; Perlmann and Troye-Blomberg, 2002). Immune responses against malaria parasite are mainly directed against the blood stages of the parasite's life cycle and are important in terms of clearing the parasite and lessening the disease (Bahouroun-Tayoun *et al*., 1990). Anti-*P. falciparum* antibodies act by blocking erythrocyte invasion by merozoites, antibody dependent cellular killing mediated by cytophilic antibodies (IgG1 and IgG3) or increased clearance of infected erythrocytes (Baruch *et al*, 1996; Groux and Gysin, 1990; Bull *et al*, 1998). All these immune effector mechanisms lead to lower parasitemia (Bahouroun-Tayoun *et al*., 1990; Crompton *et al*, 2010). Among others, the important antigens that elicit protective immunity against *P. falciparum* malaria are the asexual blood forms namely *P. falciparum* schizont antigens (PfSE), apical membrane antigen 1 (AMA1), merozoite surface proteins 1 and 2 (MSP-1 and MSP-2) and *P. falciparum* antigens expressed on the surface of infected erythrocytes or *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) (Holder *et al*, 1982; Perlman and Troye-Blomberg, 2002; Sagara *et al*, 2006; El Sahly *et al*, 2010; Esen *et al*, 2009; Hermsen *et al*, 2007). Protective immunity against *P. falciparum* infection thus depends on production of T-helper cells type 1 (Th1) characterised by production of cytokines such as interleukin (IL)-2, interferon (IFN) - γ and TNF-β. This (Th1) type of immune response clears the parasites at an early erythrocytic stage of

the infection (Helmby *et al*, 1998; Yoshida *et al*, 2000; Druilhe *et al*, 2005). It has been hypothesized that helminth infections interfere with immune responses to *P. falciparum* infection by inducing production of T-helper cells type 2 (Th2) characterised by production of cytokines such as IL-4, IL-5, IL-10, IL-13 as well as immunoglobulin E (IgE) (Maizels, 1993; Maizels *et al*, 2004; Graham 2001; Mosmann and Coffman, 1989; Spiegel *et al*, 2003). This (Th2) type of immune response favours production of non-cytophilic antibodies IgG2, IgG4 and IgM which are not protective against malaria (Maizels *et al*, 1993, Druilhe *et al*, 2005). Available evidence suggests that this helminth induced immunomodulation increases the risk of *P. falciparum* infection and disease (Tshikuka *et al*, 1996; Spiegel *et al*, 2003; Sokhna *et al*, 2004; Le Hesran *et al*, 2004; Maizels *et al*, 2004). The Th2 shift in the immune response to *P. falciparum* infection therefore triggers the production of ineffective antibodies, in contrast to the protective immune response which is associated with a Th1 response that leads to production of cytophilic antibodies IgG1 and IgG3 which are protective against malaria (Druilhe *et al*, 2005). However, some studies in West Africa have shown a protective effect of helminth infections on malaria (Briand *et al*, 2005; Lyke *et al*, 2005). In Thailand, studies have suggested that *A. lumbricoides* infection protected against the occurrence of severe malaria and acute renal failure (Nacher *et al*, 2000, Nacher *et al*, 2001; Nacher *et al*, 2002b). Studies using animal models have also produced similarly contrasting results. For example, Helmby *et al* (1998) demonstrated that co- infections with *P. chabaudi* and *S. mansoni* infections affected the immune responses to each parasite, where *S. mansoni* infection induced down-regulation of Th1 dependent immune response against *P. chabaudi* infection leading to acute and rapidly fatal clinical malaria in infected mice. Overall, these studies suggest that immunological modulation occurs in malaria and helminth co-infections.

1.2.3. Clinical consequences of co-infections

It have been observed that individuals co-infected with more than one parasite species are not only at the risk of illness associated with each parasite species, but also the risk of developing frequent and more severe disease due to interactions among the infecting parasite species (Tschikuka *et al*, 1996; Lewinsohn, 1975; Howard *et al*, 2001). Helminth infected individuals are more likely to develop clinical *P. falciparum* malaria than helminth free individuals (Nacher *et al*, 2002a; Spiegel *et al*, 2003). Similar results were observed in Kenyan children with concurrent *S. haematobium,* hookworm and *P. falciparum* infections (Stephenson *et al*, 1985). Likewise, individuals co-infected with *P. falciparum* and *S. mansoni* have been observed to develop more severe forms of hepatosplenomegaly compared to individuals infected with either of the parasites alone (Tchikuka *et al*, 1996, Booth *et al, 2004*). In Nepal, Dreyfuss *et al* (2000) observed that *P. vivax* malaria and hookworm co-infections in pregnant women were associated with more frequent attacks and severe anaemia than seen in women who harbour only one parasite infection. Furthermore, an important aspect of malaria and helminth co-infections is their joint contribution to anaemia, which affects 50% of all children and pregnant women in developing countries (Dreyfus *et al*, 2000). Malaria is a major cause of anaemia in children and pregnant women through a number of mechanisms such as destruction of red blood cells, haemolysis and dyserythropoiesis (Brooker *et al*, 2007; Davis, 2009). STH infections are associated with anaemia due to blood and iron loss into the intestinal tract (Stoltzfus *et al*, 1996). *S. mansoni* and *S. haematobium* infections also causes blood loss in faeces and urine, respectively (Davis, 2009). Furthermore, a study conducted in Nigeria demonstrated that pregnant women co-infected with *P. falciparum* and helminths produced children with lower birth weight compared to those infected with *P. falciparum* alone (Egwunyenga *et al*, 2001). More recently, studies in the Philippines has demonstrated that even at low infection intensity, multiple parasite infections enhance the risk of anaemia (Ezeamama *et al*, 2005; Ezeamama *et al*, 2008).

1.2.4. Opportunities for integrated treatment and control

Currently, several malaria and helminth control interventions exist which are proven to be efficacious, safe and cost effective. These include insecticide treated nets (ITNs), indoor residual spraying (IRS), intermittent preventive treatment and artemisinin based combination therapies for malaria (Menendez *et al*, 1997; Lengeler *et al*, 2004; Mac Otten *et al*, 2009; Sarrasat *et al*, 2008). In addition, intermittent preventive treatment in pregnant women and infants (IPTp and IPTi, respectively) are promising strategies which has been delivered to pregnant women and infants in various settings with considerable success in reducing malaria transmission and malaria related anaemia (Cisse *et al*, 2006; Sagara *et al*, 2006; Schellenberg *et al*, 2001; Chandramohan, 2005). For helminth infections, mass drug administration (MDA) using low cost, safe and efficacious antihelmintic drugs such as praziquantel and albendazole for schistosomiasis and STHs also exist (WHO, 2002; WHO, 2006). As a result, WHO recommends integration of MDA to control neglected tropical diseases including schistosomiasis and STHs and has produced guidelines which were adopted by the Schistosomiasis Control Initiative (SCI) to control schistosomiasis and STH in 6 selected countries in SSA with commendable success (WHO 2006; Fenwick, 2006; Kabatereine *et al*, 2006). Because co-infections between malaria and helminths and their clinical consequences are expected to be intensive at the age of 5-14 years, this age group should be the target for integrated control (Brooker *et al*, 2006; Brooker *et al*, 2007). School based anthelmintic delivery programmes should therefore provide an entry point for combined delivery of antimalarials, ITNs and anthelmintics (Bundy and Medley, 1992; Bundy, 1998; Mwangi *et al*, 2005; Brooker *et al*, 2007). Pregnant women are another population group at risk for malaria, schistosomiasis and helminth co-infections. IPTp using sulphadoxine pyrimethamine (SPs) is provided to pregnant women at 2nd and 3rd trimester to reduce morbidity (anaemia and low birth weight) related to pregnancy associated malaria (Shulman *et al*, 1999; Newman *et al*, 2003; Gamble *et al*, 2006). A recent study in Uganda has indicated that co-administration of albendazole and Ivermectin is safe and effective in reducing prevalence and infection intensities of STH infections in pregnant women (Ndyomugyenyi *et al*, 2008). In addition, WHO now recommends treatment of pregnant women for schistosomiasis and STH infections using praziquantel and albendazole, respectively (WHO, 2002b). Thus the findings of few published studies and what is already known on adverse effects of malaria and helminth infections in pregnancy justifies the evaluation of an integrated treatment approach using antimalarials and anthelmintics (Christian *et al*, 2004). Integration of disease control interventions is a strategy of choice when the diseases under consideration share a common population at risk, have the same technical approach to control and if collectively they exert a huge disease burden to the affected population (Utzinger and de Savigny, 2006; Hotez *et al*, 2007). There are few examples of successful integrated disease control interventions including the African Programme for Onchorcerciasis Control (APOC) in west central and east Africa which uses a community directed approach (ComDT) (WHO, 2008b), the expanded programme on immunization (EPI) and the integrated management of childhood illnesses (IMCI) (Schellenberg *et al*, 2004). Integrated control of the major tropical diseases has the potential to contribute to global poverty reduction and attainment of the millennium development goals (Hotez *et al*, 2007).

1.3. Malaria, schistosomiasis and STH infections in Tanzania

The public health importance of malaria, schistosome and STH infections in Tanzania has been reported by several studies. Malaria occurs in all parts of the country with varying levels of endemicity ranging from unstable seasonal malaria, stable malaria with seasonal variations to stable perennial malaria (Mboera and Kitua, 2001; Mboera, 2004). Malaria is the number one public health problem contributing to between 40% and 48% of all outpatients in health facilities. The disease accounts for about 40% of all hospital admissions and is responsible for more than one-third of

deaths in children under the age of five years and up to one-fifth of deaths among pregnant women (MoHSW, 2006).

With regard to helminth infections, both urinary and intestinal schistosomiasis as well as STH are present in the country (WHO, 1987; Macpherson *et al*, 1991; Lwambo *et al*, 1999). The earliest published reports on schistosomiasis in Tanzania date back to the beginning of the 20th century when Petrie reported that more than one third of men in Zanzibar were infected with *S. haematobium*. In 1909, Cook reported that some 50% of individuals examined in Mwanza had symptoms of urinary schistosomiasis (WHO, 1987). In 1911, a prevalence of 33.4% of urinary schistosomiasis was reported in Lindi, and in 1913, half of the children examined at Tunduru in the southern part of the country were infected by *S. haematobium* (Matovu and Nditi, 1980). A comprehensive review of the distribution of schistosomiasis in the country was made by McCullough in 1972. The review shows that schistosomiasis is endemic throughout Tanzania with two major zones (belts) of intensive transmission. The first zone was the Lake Victoria zone which extends all around the south-eastern and south-western hinterland for about 160 km from the lake shore. The other major zone was along the Indian Ocean coast including the islands of Zanzibar and Pemba (McCullough, 1972). More recent studies by Lwambo *et al*, 1999, Clements *et al*, 2006 and Ajanga *et al*, 2006 showed higher endemicity of schistosomiasis and STHs in the country. Several studies have also documented prevalence and intensity of malaria, schistosome and STH infections in children in Tanzania, and in Mwanza in particular (Lwambo *et al*, 1999; Magnussen *et al*, 2001; Magnussen *et al*, 2002). It has been shown that children co-infected with these parasites develop less than optimal, have reduced learning and school achievements and have increased susceptibility to other infections and anaemia (Latham *et al*, 1990; Stephenson *et al*, 1993; Stoltzfus *et al,* 1997).

1.4. Rationale of the study

Polyparasitism is common in Tanzania, and interactions that affect disease severity may occur (Lwambo *et al* 1999; Ajanga *et al*, 2006). It has been observed that malaria, schistosomiasis and the major STH infections share not only geographical distribution, but also the human hosts and individuals who harbour multiple parasite species have increased risk of severe morbidity and mortality. Though there has been a growing interest to investigate co-infections and their related clinical consequences worldwide, there have been very few longitudinal community based studies that have attempted to investigate the interactions between *P. falciparum* malaria, schistosomes and STH infections (Egwunyenga *et al*, 2001, Mwangi *et al*, 2006). Furthermore, previous studies in Mwanza have shown that prevalence of malaria, schistosomiasis and STH infections are high and therefore it is very likely co-infections and the associated interactions observed in other malaria endemic areas also occur in this area. The current study was a community based, randomized field intervention trial designed to investigate interactions between malaria, schistosomiasis, STH infections and how this interaction affects malaria infection, morbidity, anaemia and *P. falciparum* specific antibody responses among school and pre-school aged children in Magu district, North-western Tanzania.

1.5. Study objectives

1.5.1. General objective

- To provide information that will support improvements of parasitic diseases control programmes among school and pre-school children in Mwanza, Tanzania and elsewhere.

1.5.2. Specific objectives

- To elucidate the epidemiology of malaria, schistosomiasis and STH infections and anaemia among school and pre-school children in the study area.

- To determine the relationship between schistosome and STH infections and antibody response to *P. falciparum* among school and pre-school children in the study area.

- To determine the effect of an anthelmintic intervention on malaria infection, schistosome and STH infections, malaria related morbidity and on *P. falciparum* specific antibody responses among school and pre-school children in the study area.

1.6. References

Ajanga, A., Lwambo NJ., Blair L., Nyandindi U., Fenwick A., Brooker S. (2006). *Schistosoma mansoni* in pregnancy and associations with anaemia in Northwest Tanzania. *Trans Roy Soc Trop Med Hyg,* **100** (1): 59-63.

Baruch, D. I., Gormely, J. A., Ma, C., Howard, R. J., Pasloske, B. L. (1996). Plasmodium falciparum erythrocyte membrane protein 1 is a parasitized erythrocyte receptor for adherence to CD36, thrombospondin, and intercellular adhesion molecule 1. *Proc Natl Acad Sci U S A.* 1996: **93** (8): 3497 – 3502.

Bethony, J., Brooker, S., Albonico, M., Geiger. S. M., Loukas. A., Diemert, D., Hotez, P. J. (2006). Soil transmitted helminth infections: Ascariasis, tichuriasis and hookworm. *Lancet,* **367**: 1521-1532.

Booth, M., Vennervald, B. J., Kenty, L., Butterworth, A. E., Kariuki, H. C., Kadzo, H., Amaganga, C., Kimani, G., Mwatha, J. K., Otedo, A., Ouma, J. H., Muchiri, E., Dunne, D. W. (2004). Micro-geographical variation in exposure to *S. mansoni* and malaria, and exacerbation of splenomegaly in Kenyan School age children. *BMC Infect Dis*, **4:** 13.

Bouharoun-Tayoun, H,, Attanath, P., Sabchareon, A., Chongsuphajaisiddhi, T., Druilhe, P. (1990). Antibodies that protect humans against Plasmodium falciparum blood stages do not on their own inhibit parasite growth and invasion in vitro, but act in cooperation with monocytes. *J. Exp. Med*., **172** (6): 1633–1641.

Briand, V., Waiter, l., Le Hesran, J. Y., Garcia, A., Cot, M. (2005). Co-infection with plasmodium and *S. haematobium*: Protective effect of schistosomiasis on malaria in Senegalese children. *Am J. Trop. Med. Hyg*, **72**: 702-707.

Brooker, S., Clements, A. C., Hotez, P. J., Hay, S. I., Tatem, A. J., Bundy, D. A. P., Snow, R. W. (2006). The co-distribution of *Plasmodium falciparum* and hookworms among African school children. *Mal J*, **5**: 99.

Brooker, S., Akhwale, W., Pullan, R., Estambale, B., Clarke, S. E., Snow, R. W., Hotez, P. J. (2007). Epidemiology of plasmodium-helminth co-infections in Africa: Population at risk, potential impact on anaemia and prospects for combined control. *Am. J. Trop. Med. Hyg,* **77:** S88-S98.

Brooker, S and Bundy, D. A. P. (2009). Helminth infections: Soil-transmitted helminthiasis. In: Manson's tropical diseases. 22nd Ed. Sounders. UK. pp 1515- 1540.

Bull, P. C., Lowe, B. S., Kortok, M., Molyneux, C. S., Newbold, C. I., Marsh, K. (1998). Parasite antigens on the infected red cell surface are targets for naturally acquired immunity to malaria. *Nat Med*. **4** (3): 358–360.

Bundy, D. A. P. (1998). Population ecology of intestinal helminth infections in human communities *Philos. Trans. Roy. Soc. (Series B),* **321**: 405-420.

Bundy, D. A. P., Chan M. S., Savioli, L. (1995). Hookworm infection in pregnancy. *Trans Roy Soc Trop Med Hyg*, **89:** 521-521.

Bundy, D. A.P. & Medley, G. F. (1992). Immuno–epidemiology of human geohelminthiasis: ecological and immunological determinants of worm burden. *Parasitol*, **104:** S105–S119.

Bundy, D. A. P., Schaeffer, S., Jukes, S., Beegle, K, Gillespie, A., Drake, L., Seung-hee Frances Lee., Hoffman, A. M., Jones, J., Mitchel, A., Wright, C., Barcelona, D., Camara, B., Golmar, C., Savioli, L., Takeuchi, T., Sembene, M. (2006). School based health and nutrition programmes. In: Jamison, d., Claeson, M., Breman, J., Meacham, A (Eds.), Disease control priorities for developing countries. Oxford University Press, Oxford (chapter 61).

Chandramohan, D., Owusu-Agyei, S., Carneiro, I., Awine, T., Amponsa-Achiano K., Mensah, N., Jaffar, S., Baiden, R., Hodgson, A., Binka, F., Geenwood, B. (2005). Cluster randomized trial of intermittent treatment for malaria in infants in an area of high seasonal transmission in Ghana. *BMJ*, **331**: 727-733.

Chitsulo, L., Engels, D., Montresor, A., Savioli, L. (2000): The global status of schistosomiasis and its control. *Acta Trop*, **77**: 41-51.

Christian, P., Khatry, S. K., West, K. P. (2004). Antenatal anthelmintic treatment birth weight, and infant survival in rural Nepal. *Lancet*, **364**: 981-983

Cisse, B., Sokhna, C., Boulanger, D., Milet, J., Bael, H., Richardson, K., Hallet, R., Sutherland, C., Simondon, K., Simondon, F., Alexander, N., Gaye, O., Targett, G., Lines, J., Greenwood, B., Trape, J. F. (2006). Seasonal intermittent preventive treatment with artesunate and sulphadoxine-pyrimethamine for prevention of malaria in Senegalese children: A randomized, placebo controlled, double blind trial. *Lancet*, **367**: 659-667.

Clarke, S., Brooker, S., Njagi, K., Njau, E., Estambale, B., Muchiri, E. & Magnussen, P. (2004). Malaria morbidity amongst schoolchildren living in two areas of contrasting transmission in western Kenya. *Am J Hyg Trop Med*, **71**: 732–738.

Clements, A. C., Lwambo, N. J., Blair, L., Nyandindi, U., Kaatano, G., Kinung'hi, S., Webster, J. P., Fenwick, A., Brooker, S. (2006). Bayesian spatial analysis and disease mapping: tools to enhance planning and implementation of a schistosomiasis control programme in Tanzania. *Trop Med Int Health*. **11:** 490-503.

Cohen, S., McGregor, I. A., Carrington, S. (1961). Gammaglobulin and acquired immunity to human malaria. *Nature,* **192**: 733–737.

Cox, F. E. G. (2001). Concomitant infections, parasites and immune responses. *Parasitol*, **122:** 23-38.

Crompton, P. D., Pierce, S. K., Miller, L. H. (2010). Advances and challenges in malaria vaccine development. *J Clin Invest*, **120** (12): 4068- 4178.

Davis, A. (2009). Helminth infections: Schistosomiasis. In: Manson's tropical diseases. 22nd Ed. Sounders. UK. pp 1425- 1460.

De Silva, N. R., Brooker, S., Hotez, P, J., Montresor, A., Engels, D., Savioli, L. (2003). Soil transmitted helminth infections: Updating the global picture. *Trends Parasitol*, **19**: 547-551.

De Silva, N. R. (2003). Impact of mass chemotherapy on the morbidity due to soil transmitted nematodes. *Acta Trop*, **86:** 197-214.

Dreyfuss, M. L., Stoltzfus, R. J., Shrestha, J. B., Pradhan, E. K., Leclerc, S. C., Khatri, S. K., Shresha, S. R., Katz. J., Albonico, M., West, K. P. (2000). Hookworms, malaria and vitamin A deficiency contributes to anaemia and iron deficiency among pregnant women of Nepal. *J Nutr,* **130:** 2527-2536.

Druilhe, P., Tall, A., Sokhna, C. (2005). Worms can worsen malaria. Towards a new means to roll back malaria? *Trends Parasitol*, **21:** 359-362.

Egwunyenga, O. A., Ajayi, J. A., Nmorsi, O. P. G., Duhlinska-Popova, D. D. (2001). Plasmodium/intestinal helminth co-infections among pregnant Nigerian women. *Mem. Inst. Oswaldo Cruz*, **96** (8): 1055-1059.

El Sahly, H. M., Patel, S. M., Atmar, R. L., Lanford, T. A., Dube, T., Thompson, D., Sim, B. K., Long, C., Keitel, W. A. (2010). The safety and immunogenicity of recombinant EBA 175-RII NG malaria vaccine in healthy adults living in a non-endemic area. *Clin Vaccine Immunol*. 17 (10): 1552–1559.

Engels, D., Chitsulo, L., Montresor, A., Savioli, L. (2002): The global epidemiological situation of schistosomiasis and new approaches and new approaches to control and research. *Acta Trop*, **82**: 139-146.

Esen, M., Kremsner, P. G., Schleucher, R., Gässler, M., Imoukhuede, E. B., Imbault, N., Leroy, O., Jepsen, S., Knudsen, B. W., Schumm, M., Knobloch, J., Theisen, M., Mordmüller, B. (2009). Safety and immunogenicity of GMZ2 a MSP3-GLURP fusion protein malaria vaccine candidate. *Vaccine*. **27** (49): 6862–6868.

Ezeamama, A. E., Friedman J. F., Olveda R. M., Acosta L. P., Kurtis J. D., Mor V., McGarvey, S. T. (2005). Functional significance of low intensity polyparasite helminth infections in anaemia. *J. Inf. Dis*, **192:** 2160-2170.

Ezeamama, A. E., McGarvey, S. T., Acosta L. P., Zierler S., Manalo D. L., Wu H. W., Kurtis J. D., Mor V., Olveda R. M., Friedman J. F. (2008). The synergistic effect of concomitant schistosomiasis, hookworm, and trichuris infections on children's anemia burden. *PLoS Negl Trop Dis,* **2** (6): e245.

Fenwick, A. (2006). New initiatives against Africa's worms. *Trans Roy. Soc. Trop. Med. Hyg*, **100:** 200-207.

Gamble, C., Ekwaru, J. P., te Kuile, F. O. (2006). Insecticide treated nets for prevention of malaria in pregnancy. *Chocrane Data Syst Rev,* **2**: CD003755.

Geissler, P. W., Mwaniki D., Thiongo F., Friis H. (1998). Geophagia as a risk factor for geohelminth infections: a longitudinal study of Kenyan primary schoolchildren. *Trans Roy Soc Trop Med Hyg,* **92**: 7-11.

Groux, H and Gysin, J. (1990). Opsonization as an effector mechanism in human protection against asexual blood stages of Plasmodium falciparum: functional role of IgG subclasses. *Res Immunol*. **141** (6): 529 – 542.

Graham, S. P., Trees, A. J., Collins, R. A., Moore, D. M., Guy, F. M., Taylor, M. J and Bianco, A. E. (2001). Down-regulated lymphoproliferation coincides with parasite maturation and with the collapse of both gamma interferon and interleukin-4 responses in a bovine model of onchocerciasis. *Infect Immun*, **69:** 4313–4319.

Helmby, H., Kullberg, M., Troye-Blomberg, M. (1998). Altered immune responses in mice with concomitant *Schistosoma mansoni* and *Plasmodium chabaudi* infections. *Infect Immun*, **66** (11): 5167-5174.

Hermsen, C. C., Verhage, D. F., Telgt, D. S., Teelen, K., Bousema, J. T., Roestenberg, M., Bolad, A., Berzins, K., Corradin, G., Leroy, O., Theisen, M., Sauerwein, R. W. (2007). Glutamate-rich protein (GLURP) induces antibodies that inhibit in vitro growth of Plasmodium falciparum in a phase 1 malaria vaccine trial. *Vaccine,* 25 (15): 2930–2940.

Holder, A. A and Freeman, R. R. (1982). Biosynthesis and processing of a P. falciparum schizont antigens recognised by immune serum and monoclonal antibody, *J. Exp. Med*, **156**: 1528-1538.

Hotez, P. J., Brooker, S., Bethony, J. M. (2004). Current concepts: Hookworm infection. *N Engl J Med,* **351** (8): 799-808.

Hotez, P., Molyneux, D. H., Fenwick, A., Kumaresan, J., Sachs, S. E., Sachs, J. D., Savioli, L. (2007). Control of neglected tropical diseases. *N. Eng. J. Med*. **357** (10): 1018-1026.

Howard, S. C., Donnelley, C. A., Chan, M.-S. (2001). Methods for the estimation of associations between multiple species parasite infections. *Parasitol*, **122:** 233-251.

Kabatereine, N. B., Tukahebwa E., Kazibwe F., Namwangi, H., Zaramba S., Brooker S., Stothard JR., Kamenka C., Whawell S., Webster J. P., Fenwick A. (2006). Progress towards countrywide control of schistosomiasis and soil-transmitted helminthiasis in Uganda. *Trans. Roy. Soc. Trop. Med. Hyg*, **100:** 208-215.

Kimbi, H. K., Awah, N. W., Ndamukong, K. J., Mbuh, J. V. (2005). Malaria infections and its consequences in school children. *East African Med J*, **82:** 92-97.

Latham, M. C., Stephenson, L. S., Kurz, K. M., Kinoti, S. N (1990). Metrifonate or praziquantel treatment improves physical fitness and appetite of Kenyan schoolboys with Schistosoma haematobium and hookworm infections. *Am. J. Trp. Med. Hyg*, **43:** 170-179.

Le hesran, J. Y., Akiana, J., Ndiaye, E. H. M., Dia, M., Senghor, P., Konate, L. (2004). Severe malaria attack is associated with high prevalence of Ascaris lumbricoides infection among children in rural Senegal. *Trans. Roy. Soc. Trop Med. Hyg*, **98:** 397-399.

Lengeler, C. (2004). Insecticide-treated nets for malaria control: real gains. *Bull World Health Organ,* **82** (2): 85-91.

Lewinsohn, R. (1975). Anaemia in mice with concomitant *S. Mansoni* and *P. berghei yoelii* infection. *Trans Roy. Soc Trop Med Hyg* **69**, (1), 1975, 51-56.

Lwambo, N. J., Siza J. E., Brooker S., Bundy D.A., Guyatt H. (1999). Patterns of concurrent hookworm infection and schistosomiasis in schoolchildren in Tanzania. *Trans Roy Soc Trop Med Hyg,* **93:** 497-502.

Lyke, E. K., Dicko, A., Bado, B., Sangare, L., Kone, A., Coulibaly, D., Guindo, A., Traore, K., Daou, M., Diarra, I., Sztein, M. B., Plowe, C. V., Doumbo, O. K. (2005). Association of *Schistosoma haematobium* infection with protection against acute plasmodium falciparum malaria in Malian children. *Am. J. Trop Med. Hyg*, **73** (6): 1124-1130.

Otten, M., Aregawi, M., Were, W., Karema, K., Medin, A., Bekele, W., Jima, D., Gausi, K., Komatsu, R., Korenromp, E., Low-Beer, D., Grabowsky, M. (2009). Initial evidence of reduction of malaria cases and dealhs in Rwanda and Ethiopia due to rapid scale- up of malaria prevention and treatment. *Mal J* , **8**: 14.

Malaria Immunology (2002) Eds. Perlmann, P and Troye-Blomberg, 2nd edition, Chem Immunol **80**, 229-242.

Macpherson, C. N. L., Kilama, W. L., Kihamia, C. M. (1991). Miscellaneous parasitic infections: Nematodes. In: Mwaluko, G. M. P., Kilama, W. L., Mandara, M. P., Murru, M., Macpherson, C. N. L. (1991). Health and Disease in Tanzania. (Eds.). HarperCollinsAcademic, London. pp 144-158.

Magnussen, P., Ndawi, B., Sheshe, A. K., Byskov, J., Mbwana, K. (2002). Prevalence and morbidity of urinary schistosomiasis among school children in the Mwera Division of Pangani district, Tanzania. *Ann Trop Med Parasitol*, **96** (8): 843-848.

Magnussen, P., Ndawi, B., Sheshe, A. K., Byskov, J., Mbwana, K., Christensen, N. O. (2001). The Impact of a school health programme on the prevalence and morbidity of urinary schistosomiasis in Mwera Division, Pangani District, Tanzania. *Trans Roy Soc Trop Med Hyg*, **95** (1): 58-64.

Magnussen, P. Ndawi, B., Sheshe, A. K., Byskov, J., Mbwana, K. (2001). Malaria diagnosis and treatment administered by teachers in primary schools in Tanzania. *Trop. Med. Int. Health,* **6**: 273-279.

Maizels, R. M., Bundy, D. A., Serkirk, M. E., Smith, D. F., Anderson, R. M. (1993). Immunological modulation and evasion by helminth parasites in human populations. *Nature*, **365:** 797-805.

Maizels, R. M., Balic, A., Gomez-Escobar N., Nair M., Taylor, M. D, Allen J. E. (2004). Helminth parasites–masters of regulation. *Immunol Rev.* **201:** 89–116.

Marsh, K (2002). Immunology of malaria. In: Warrel, D and Gilles, H. M. (2002). Essential malariology. 4th Eddition, Arnold Publishers, London: UK. pp 252-267.

Matovu, D. B and Nditi, H. P (1980). The distribution and intensity of schistosomiasis in Tanzania (Mainland). In: Annual scientific conference of the medical association of Tanzania. Dar-Es-Salaam, September 1980, 12 p.

Mboera, L. E. G and Kitua, A. Y (2001). Malaria epidemics in Tanzania; *African J Health Sci,* **8**: 14-18.

Mboera, L. E. G (2004). Environmental and socio-economic determinants of malaria epidemics in highlands of Tanzania. *Tanzania Health Res Bull*, **6:** 11-17.

McCullough, F. S. (1972). The distribution of *S. mansoni* and *S. haematobium* in East Africa. *Trop Geog Med*, **24:** 199-207.

Menendez, C., Kahigwa, E., Hirt R., Vounatsou P., Aponte JJ, Font, F., Acosta C, J., Schellenberg D. M,, Galindo, C. M, Kimario, J., Urassa H., Brabin, B, Smith, T. A, Kitua, A.Y, Tanner M, Alonso P. L .(1997). Randomized placebo-controlled trial of iron supplementation and malaria chemoprophylaxis for prevention of severe anaemia and malaria in Tanzanian infants. *Lancet*, **350:** 844-850.

MoHSW (2006). *Annual Health Statistics Abstract*. Ministry of Health and Social Welfare, Dar-Es-Salaam, United Republic of Tanzania.

Mosmann, T. R. and Coffman, R. L. (1989). TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Ann Rev Immunol*, **7**: 145–173.

Mwangi, T. W., Bethony., J. M., Brooker, S. (2006). Malaria and helminth interactions in humans: An epidemiological viewpoint. *An. Trop. Med. Parasitol*, **100** (7): 551-570.

Nacher, M., Singhasivanon, P., Yimsamran, S., Manibunyong, W., Thanyavanich, N., Wuthisen, P, Looareesuwan, S (2002a). Intestinal helminth infections are associated with increased incidence of *Plasmodium falciparum* malaria in Thailand. *J Parasitol*, **88** (1): 55-58.

Nacher, M., Gay, F., Singhasivanon, P., Krudsood, S., Treeprasertsuk, S., Mazier, D., Vouldoukis, I. & Looareesuwan, S. (2000). Ascaris lumbricoides infection is associated with protection from cerebral malaria. *Par Immunol,* **22:** 107–113.

Nacher, M., Singhasivanon, P., Silachamroon, U., Treeprasertsuk, S., Vannaphan, S., Traore, B., Gay, F. & Looareesuwan, S. (2001). Helminth infections are associated with protection from malaria-related acute renal failure and jaundice in Thailand. *Am J Trop Med Hyg*, **65:** 834–836.

Nacher, M., Singhasivanon, P., Traore, B., Vannaphan, S., Gay, F., Chindanond, D., Franetich, J. F., Mazier, D. & Looareesuwan, S. (2002b). Helminth infections are associated with protection from cerebral malaria and increased nitrogen derivatives concentrations in Thailand. *Am J Trop Med Hyg*, **66:** 304–309.

Ndyomugyenyi, R., Kabatereine, N., Olsen, A., Magnussen P (2008). Efficacy of ivermectin and albendazole alone and in combination for treatment of soil-transmitted helminths in pregnancy and adverse events: a randomized open label controlled intervention trial in Masindi district, western Uganda. *Am J Trop Med Hyg*, **79** (6): 856-863.

Newman, R. D., Parise, M. E., Slutsker, L., Nahlen, B., Steketee, R. W. (2003). Safety, efficacy and determinants of effectiveness of antimalarial drugs during pregnancy: implications for prevention programmes in *Plasmodium falciparum*-endemic sub-Saharan Africa. *Trop Med Int Health,* **8***:* 488–506.

Patney, T. N., Andrews, R. H. (1998). Multiparasite communities in animals and humans: frequency, structure and pathogenic significance. *Int J Parasitol,* **28** (3): 377-393.

Rollingson, D and Southgate, V (1987). The genus schistosoma: A taxonomic appraisal. In: The biology of schistosomes, from genes to latrines. Ed by Rollingson D and Sympson A. J. G Academic press Ltd, London.

Sabchareon, A., Burnouf, T., Ouattara, D., Attanath, P., Bouharoun-Tayoun H,, Chantavanich, P., Foucault, C., Chongsuphajaisiddhi, T., Druilhe, P. (1991). Parasitologic and clinical human response to immunoglobulin administration in falciparum malaria. *Am J Trop Med Hyg*. 1991; **45** (3): 297–308.

Sagara, I., Dicko, A., Djimde, A., Guindo, O., Kone, M., Tolo, Y., Thera, M. A., Sogoba, M., Fofana, M., Ouattara, A., Sissoko, M., Jansen, H. F., Doumbo, O. K. (2006). A randomized trial of artesunate-sulfamethoxypyrazine-pyrimethamine versus artemether-lumefantrine for the treatment of uncomplicated Plasmodium falciparum malaria in Mali. *Am. J. Trop. Med. Hyg,* **75**: 630-636.

Sagara, I., Ellis, R. D., Dicko, A., Niambele, M. B., Kamate, B., Guindo, O., Sissoko, M. S., Fay, M. P., Guindo, M. A., Kante, O., Saye, R., Miura, K., Long, C., Mullen, G. E., Pierce, M., Martin, L. B., Rausch, K., Dolo, A., Diallo, D. A., Miller, L. H., Doumbo, O. K. (2009). A randomized controlled phase 2 trial of the blood stage AMA1-C1/Alhydrogel malaria vaccine in children in Mali. *Vaccine,* **27** (23): 3090–3098.

Sarrassat, S., Senghor, J., Le Hesran, J. Y. (2008). Trends in malaria morbidity following the introduction of artesunate plus amodiaquine combination in M'lomp village dispensary, Southwest Senegal. *Mal J*, **7**: 215.

Schellenberg, D., Menendez, C., Kahigwa, E., Aponte, J., Vidal, J., Tanner, M., Mshinda, H., Alonzo, P. L. (2001). Intermittent treatment for malaria and anaemia control at time of routine vaccination in Tanzanian infants: A randomized placebo controlled trial. *Lancet*, **357**: 1471-1477.

Schellenberg, A. J. R., Adam, T., Mshinda, H., Masanja, H., Kabadi, G., Mukasa, O., John, T., Charles, S., Nathan, R., Wilczynska, K., Mgalula, L., Mbuya, C., Mswia, R., Manzi, F., de Savigny, D., Schellenberg, D., Victora, C. (2004). Effectiveness and cost of facility-based Integrated Management of Childhood Illness (IMCI) in Tanzania. *Lancet,* **364:** 1583-1594.

Shulman, C. E., Dorman, E. K., Cutts, F., Kawuondo, K., Bulma, J. N., Peshu, N., Marsh, K. (1999). Intermittent sulphadoxine-pyrimethamine to prevent severe anaemia secondary to anaemia in pregnancy. A randomized placebo controlled trial. *Lancet*, **353:** 632-636.

Snow, R. W., Omumbo, J. A., Lowe, B., Molyneux, C. S., Obiero, J. O., Palmer, A., Weber, M. W., Pinder, M., Nahlen, B., Obonyo, C., Newbold, C., Gupta, S. & Marsh, K. (1997). Relation between severe malaria morbidity in children and level of Plasmodium falciparum transmission in Africa. *Lancet,* **349:** 1650–1654.

Snow, R. W and Gilles, H. M (2002). The epidemiology of malaria. In: Bruce Chwatt's Essential Malariology. 4th Edition. Edited by: D. A. Warrell, H. M. Gilles. Arnold Publishers, London; 2002: 85-106.

Snow, R. W., Guerra, C. A., Noor, A. M., Myint, H. Y., Hay, S. I. (2005). The global distribution of clinical episodes of Plasmodium falciparum malaria*. Nature*, **434:** 214–217.

Snow, R W., Guera, C. A., Noor, A. M., Myint, H. Y., Hay, H. I (2005). The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature*, **434:** 214-217.

Sokhna, C., Le Hesran, J. Y., Mbaye, P. A., Akiana, J., Camara, P., Diop, M., Ly, A., Druilhe, P. (2004). Increased malaria attacks among children presenting concomitant infections by *S. mamsoni* in Senegal. *Mal J*, **3**: 43.

Spiegel, A., Tall, A., Raphenon, G., Trape, J. F., Druilhe, P. (2003). Increased frequency of malaria attacks in subjects co-infected by intestinal worms and *Plasmodium falciparum* malaria. *Trans. Roy. Soc. Trop. Med. Hyg*, **97:** 198-199.

Stephenson, L. S., Latham, M. C., Kinoti, S. N., Pertet, A. (1993). Physical fitness, growth and appetite of Kenyan school children with hookworm, T*richuris trichiura and Ascaris lumbricoides* infections are improved four months after a single dose of albendazole. *J Nutr*, **123:** 1036-1046.

Stephenson, L. S., Latham, M. C., Kurz, K. M., Kinoti, S. N., Oduori, M. L., Crompton, D. W (1985). Relationships of Schistosoma haematobium, hookworm and malarial infections and metrifonate treatment to hemoglobin level in Kenyan school children. *Am J Trop Med Hyg*, **34** (3): 519 - 528.

Stephenson, L. S., Latham, M C, Ottesen, E. A. (2000). Malnutrition and parasitic helminth infections. *Parasitol* **121:** S23-38.

Stoltzfus, R. J., Albonico, M., Chwaya, H. M., Savioli, L., Tielsch, J., Schulze, K., Yip, R. (1996). Haemoquant determination of hookworm related blood loss and its role in iron deficiency in African children. *Am. J. Trop. Med. Hyg,* **55:** 399-404.

Stoltzfus, R. J., Chwaya, H. M., Tielsch, J., Schulze, K., Albonico, M., Savioli, L. (1997). Epidemiology of iron deficiency anaemia in Zanzibari schoolchildren: The importance of hookworms. *Am J Clin Nutr,* **65**: 153-159.

Sturrock, R. F (1987). Biology and ecology of human schistosomes. In: Mahmoud, A. A. F (1987). Clinical and tropical medicine and communicable diseases. International practice and research. Vol 2. No. 2. London: Bailliere Tindall, August 1987, pp 249-259.

Tschikuka, J. G., Scott, M. E., Gray-Donald, K., Kalumba, O. N. (1996). Multiple infection with plasmodium and helminths in communities of low and relatively high socio-economic status. *Ann Trop Med Parasitol*, **90** (3): 277-293.

Ukoli, F. M. A. (1984). Human intestinal nematodes. In: F. M. A Ukoli. Introduction to parasitology in tropical Africa. (Eds.). John Wiley & Sons Ltd. Norwich, UK. Pp 227-262.

Utzinger, J and Keiser, J. (2004). Schistosomiasis and Soil transmitted helminthiasis: Common drugs for treatment and control. *Expert Opin. Pharma*, **5**: 263-285.

Utzinger J, de Savigny D. (2006). Control of neglected tropical diseases: integrated chemotherapy and beyond. *PLoS Med,* **3** (5): e112.

Van der Werf M. J., de Vlas, S. J., Brooker, S., Looman, C. W., Nagelkerke, N. J., Habbema, J. D., Engels, D (2003). Quantification of clinical morbidity associated with schistosome infection in Sub Saharan Africa. *Acta Trop*, **86**: 125-139.

Warren, K. (1987). Schistosomiasis: Past, Present and Future. Mem. Inst. Oswaldo Cruz, 82, suppl. IV, 15-29.

White, N. J (2009). Protozoan infections: Malaria. In: Manson's tropical diseases. 22nd Ed. Saunders. UK. pp 1201- 1300.

WHO (2002). Prevention and control of schistosomiasis and soil-transmitted helminthiasis. *Report of WHO Expert Committee*. WHO, Geneva. 1-24.

WHO (2002b). Report of the WHO informal consultation on the use of praziquantel during pregnancy/lactation and albendazole/mebendazole in children under 24 months. WHO, Geneva, 8 – 9 April 2002.

WHO (2006). Schistosomiasis and soil transmitted helminth infections-A preliminary estimate of the number of children treated with albendazole or mebendazole. *Weekly epidemiological record*, **81:** 145-164.

WHO (2008). Estimated burden of malaria in 2006. *World malaria report 2008*, WHO Geneva. pp 9-15.

WHO (2006). Preventive chemotherapy in human helminthiasis. Coordinated use of antihelmintic drugs in control interventions. A manual for health professionals and programme managers. World Health Organization, Geneva. pp 1-62.

WHO (2008b). Community directed interventions (CDI) for major health problems in Africa. WHO, Geneva, 2008.

WHO (1987). Atlas of the global distribution of schistosomiasis. OMS-WHO, 1987. 233-242

Yoshida, A., Maruyama, H., Kumagai, T., Amano, T., Kobayashi, F., Zhang, M., Himeno, K., Ohta, N (2000): *Schistosoma mansoni* infection cancels the susceptibility to *Plasmodium chabaudi* through induction of type 1 immune responses in A/J mice. *Int Iimmunol* **12** (8): 1117-1125.

Chapter 2: Methodology

2.1. Study area and population

The study was conducted in Magu district, one among 8 districts of Mwanza region North-western Tanzania. The district headquarter is located about 65km from Mwanza city along the Mwanza – Nairobi road. Magu district lies between 2^0 10' and 2^0 50' South of Equator and 33^0 and 34^0 East of Greenwich. It has an area of 3075km^2 of which 1725km^2 (56.1%) is covered by Lake Victoria waters. The district is divided into 27 wards and 125 villages. Topographically, the district consists of low lying land with wide plains along the Lake Victoria basin that comprises 12 out of the 27 wards. The rest of the district is highland area composed of isolated hills and ridges. Mean temperature ranges from 18^0C to 20^0C during the rainy season and 26^0C to 30^0C during the dry season. Rainfall is bimodal with the short rains between October to December and heavy rainfall between March and May. Mean annual rainfall ranges from 700m to 1000mm. The district has a population of 416,113 people of whom 202,077 (48.6%) are males (NBS, 2003). The district has 44 health facilities of which 2 are hospitals, 5 health centres and 37 are dispensaries. According to hospital records, malaria remains the number one cause of hospital admissions and child morbidity and mortality in the district. Malaria transmission occurs throughout the year with peaks during the two rain seasons. Magu district has many water bodies including small and large rivers, swamps and ponds particularly in areas lying in the Lake Victoria basin which are ideal for snail habitats and mosquito breeding. The district is hyper- to holoendemic for malaria with transmission occurring throughout the year (Massaga, 2003). The predominant ethnic group in Magu district is the Wasukuma who practise subsistence farming (animal husbandry and crops) and fishing in Lake Victoria. The study involved school and pre-school children aged between 3 -13 years in 6 selected primary schools.

Figure 2. 1 Administrative map of Tanzania showing the location of Mwanza region and the location of Magu district within Mwanza region

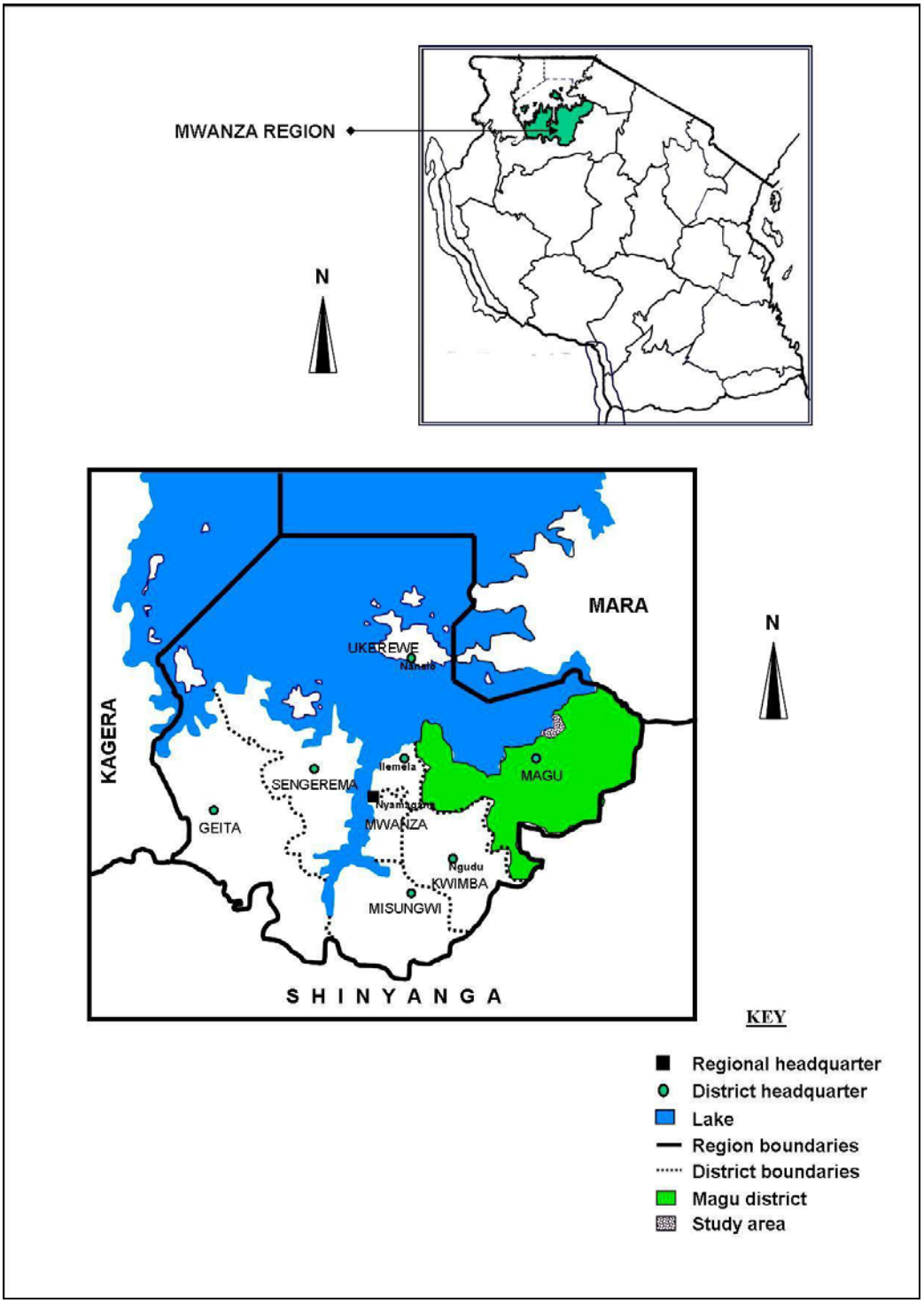
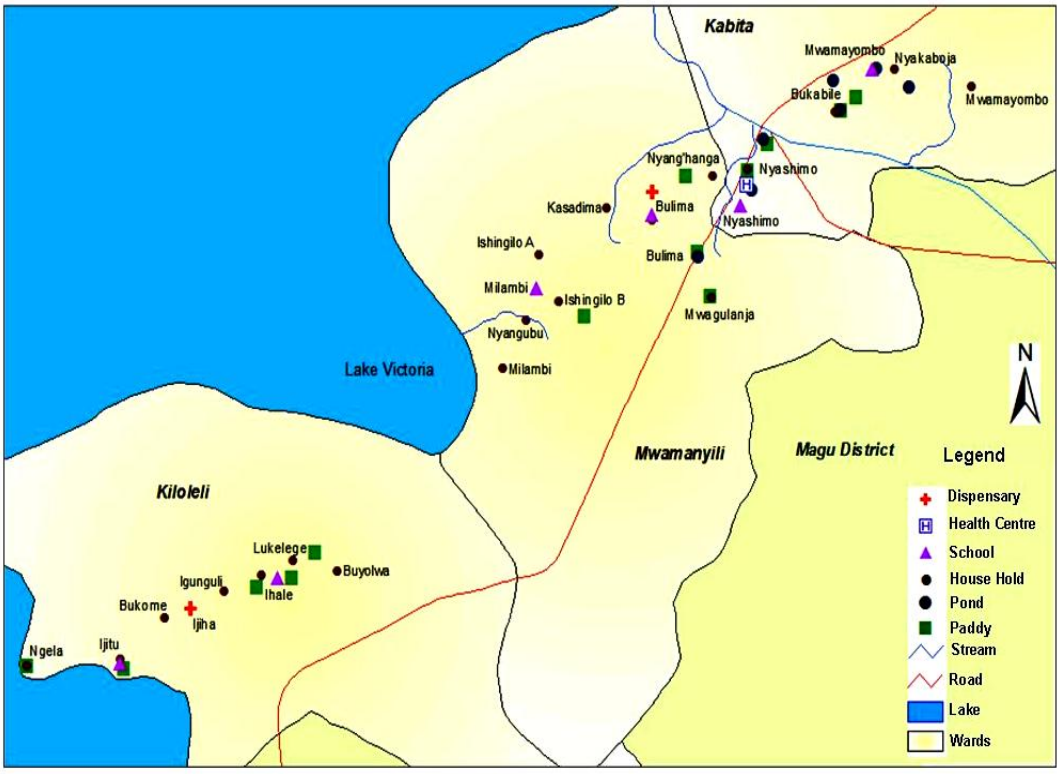


Figure 2. 2 Map of the study area showing the 6 selected schools and health facilities



2.2. Study design and sampling methods

2.2.1. Study design

The study was a prospective randomized controlled anthelmintic intervention trial with an initial baseline survey and two follow up surveys at 12 months and 24 months after baseline. The baseline survey was conducted between October and November 2006 during which 1615 children were examined. All children who were infected were treated according to national guidelines. After the baseline survey, a total of 765 children who were infected with either *S. mansoni* or *S. haematobium* or both were selected and randomized to two cohorts, which were treated with two different treatment regimens. The first cohort of 394 children (the intervention group) was treated with praziquantel 40mg/kg and albendazole 400mg four times a year at three months interval. The second cohort of 371 children (the control group) was treated with praziquantel 40mg/kg and albendazole 400mg once a year. The first and second follow up surveys were conducted between October and November 2007 and 2008, respectively. During the baseline and follow up surveys parasitological examination of stool, urine and blood samples was performed. Assessment of blood samples for Hb concentrations was carried out. Serum samples were prepared from blood samples and analysed for specific antibodies against *P. falciparum.* Clinical and ultrasound assessment for organ pathology (size and consistency) of the spleen and liver was also performed.

2.2.2. Sample size determination

The sample size was estimated by taking haemoglobin (Hb) concentration as the reference parameter and aiming to detect a difference in mean Hb concentrations of 5g/L between the intervention and control group at 5% significance level, 95% confidence interval and 90% power of the test. By applying the formula on comparison of means (Kirkwood and Sterne, 2003), and assuming a loss to follow up of 20% during the two years of the intervention, the minimum sample size required was estimated to be 310 children per each group, making a total of 620 children.

2.2.3. Selection of schools and children

A purposive sampling method was used to select the primary schools. Schools selected were those which are located close to each other and close to the shores of Lake Victoria where transmission of schistosomiasis, STH and malaria is high. Communities surrounding the selected schools are mainly involved in subsistence farming of maize, cotton, millet, sweat potatoes, rice and cassava. They also practice small scale animal husbandry (cattle, goat, sheep and chicken). Few people have small scale businesses. Because of close proximity to the lake shore, communities also practice fishing and also obtain water for most of their domestic needs from the lake. Six primary schools were selected namely Mwamayombo, Nyashimo, Bulima, Milambi, Ihale and Ijitu. The schools selected were located within a distance of 15 to 30 kilometres North-East of Magu town along the main road running from Mwanza city to Nairobi. In each primary school, all children in grade one and preparatory grade (nursery) were selected. Pre-school children aged 5 years and above were also enrolled from the surrounding communities.

2.2.3.1. Inclusion criteria

- Primary schools in Magu district, close to each other and close to the shores of Lake Victoria.
- In each selected school, pupils in grade one and preparatory grade were included in the study.

2.2.3.2. Exclusion criteria

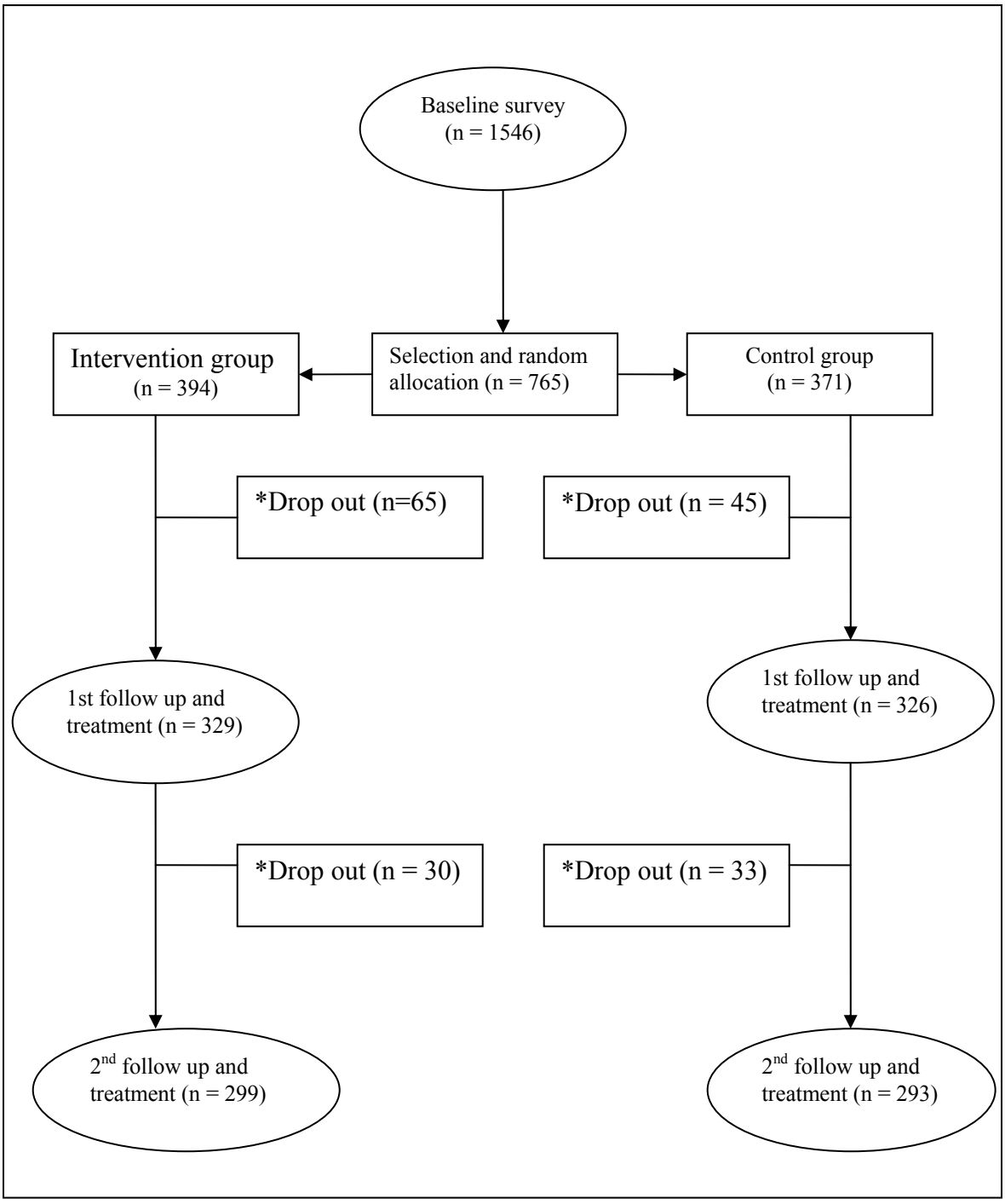
- Children whose parents or legal guardians did not provide informed consent.
- Children who had received anthelmintic treatment before the baseline study.

2.2.4. Randomization and treatments

The objective of the antihelmintic intervention study was to determine the effect of the anthelmintic intervention on malaria malaria infection, malaria related morbidity including anaemia and on *P. falciparum* specific antibody responses among school and pre-school children. Using randomly generated numbers, the 765 children who were initially infected with either *S. mansoni* or *S. haematobium* or both were randomized into either an intervention group (394 children) or a control group (371 children). The intervention group was treated with praziquantel 40mg/kg and albendazole 400mg four times a year at three months interval while the control group was treated with praziquantel 40mg/kg and albendazole 400mg once a year. All treatments took place at the school premises and were administered by a qualified medical doctor assisted by a nurse and trained school teachers. Before taking the drugs, children were given a piece of bread and a soft drink (juice) to minimise side effects of praziquantel. While albendzole was given as a single dose at a dosage rate of 400mg, praziquantel was administered according to weight. All drugs were taken orally together with clean drinking water and swallowing of tablets was done under supervision.

After taking the drugs, children were allowed to rest for a period of 30-60 minutes during which side effects related to praziquantel treatment were monitored.

Figure 2.3 Flow diagram of the study



*Reasons for dropout 1st follow up: 57 children (51.8%) migrated from the study area, 35 children (31.8%) had second sample missing, 6 children (5.5%) reasons were unknown, 5 children (4.5%) were sick during the day of the survey. Other reasons included consent withdrawal (3 children), exclusion from the study (3 children) and death (1 child).

*Reasons for drop out 2nd follow up: 34 children (54%) migrated from study area, 21 children (33.3%) had second sample missing, 4 children (6.3%) reasons for drop out unknown and 4 children (6.3%) were sick during the day of the survey.

Out of the 765 children who were included in the longitudinal study, 655 and 592 were available during the first and second follow up survey and treatments, respectively, making an overall coverage of 77.4% (Figure 2.3).

2.3. Monitoring of clinical malaria attacks

During the intervention, clinical malaria attacks were monitored at each primary school assisted by school teachers. The teachers were trained on how to make presumptive diagnosis and treatment of malaria cases, collect finger prick blood, prepare and preserve Giemsa stained blood smears and keep records. A register of malaria cases was established at each primary school. A laboratory technician visited each school on a weekly basis to collect the Giemsa stained blood smears and all information recorded and send these to the laboratory at Mwanza Medical Research Centre for confirmation. School children found with febrile illness were provided with first line antimalarials or referred to nearby health facilities according to national guidelines on malaria treatment. A malaria episode (case) was defined as a child presenting with axillary temperature $\geq$ 37.5 ^{0}C plus a positive thick blood smear for malaria parasites in the absence of signs for other infectious diseases.

2.4. Ethical considerations

The project proposal was approved by the Medical Research Coordination Committee (MRCC) of the National Institute for Medical Research (NIMR), Tanzania (Reference No. NIMR/HQ/R.8a/Vol. IX/355). The study was also reviewed, commented on and accepted as ethical by the Danish National Committee on Biomedical Research Ethics. Before commencement of the project, the principle investigator and his research team conducted meetings with leaders and head teachers of all selected villages during which the objectives of the study was explained. The village leaders then convened village meetings during which sensitization of the communities was carried out. During these meetings, the objectives of the study including the study procedures to be followed, samples to be taken, study benefits and potential risks and discomforts were explained. Informed consent for children to participate in the study was sought from parents and legal guardians after they have been clearly informed about the study. Children were informed of their right to refuse to participate in the study and to withdraw at any time during the study without jeopardizing their right of access to other health services. Invasive procedures such as collection of blood samples were fully explained to parents and children and were carried out using sterile disposable materials. Furthermore, all processes involving collection, processing, preservation and examination of samples were carried out by trained and authorised personnel. All children found infected with any of the parasites *S. mansoni, S. haematobium,* soil-transmitted helminthiasis and *P. falciparum* and those found with ailments not targeted by the project were treated free of charge according to national guidelines. Study identification numbers were used instead of children names and information collected was kept confidential. Feedback to the study population in the form of dissemination workshops was conducted during the course of the study.

2.5. Data collection methods

2.5.1. Clinical examination

Clinical examinations for all children were performed by an experienced physician. Examination was performed with children lying on an examination bed with their knees bent in order to relax the abdominal muscles in line with a procedure described in detail by Vennervald *et al*, 2004. Briefly, measurements recorded included liver tenderness, presence of any palpable liver irregularities, the extension of the left liver lobe beneath the sternum measured in centimetres in the mid-sternal line

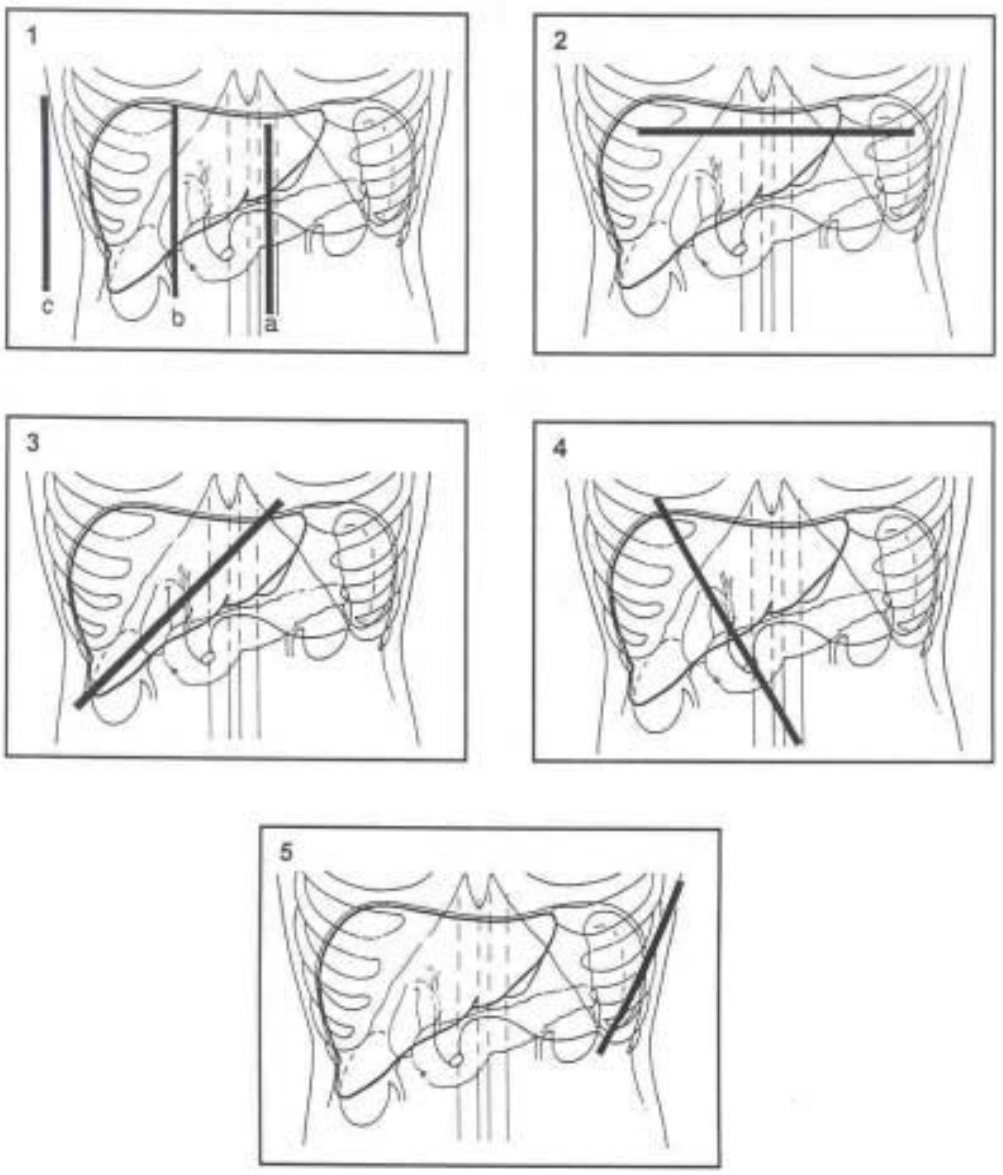
(MSL), the extension of the right liver lobe beneath the rib cage, measured in centimetres in the right mid-clavicular line (MCL) and the extension of the spleen below the rib cage, measured in centimetres in the left mid-clavicular line (MCL) and left mid-axillary line (MAL) using a measuring tape. Other measurements included the consistency of the liver and spleen graded as not palpable, soft, firm or hard. Clinical signs of portal hypertension, including ascites and umbilical collaterals, and other findings such as abdominal swellings or scars were also recorded. Anthropometric measures (weight in kilograms and height in centimetres) and body temperature (^{0}C) of each child was also recorded.

2.5.2. Ultrasound examination

All children were examined using a potable ultrasound machine (Aloka Sonocamera SSD-500 with 3.5 MHz curvilinear probe) which was supplied with power from a portable generator. A suitable jelly was used for coupling between the transducer and the skin. Examinations were performed by two experienced sonographers with extensive field experience of examination for *S. mansoni* and *S. haematobium*. The examinations were performed using standard scans according to the Niamey protocol (Richter *et al*, 2000). This involved measurements of the size of the left liver lobe (Liver PSL) by longitudinal liver scan. The portal vein diameter (PVD) was measured at the point of entrance of the portal vein into the liver. Presence or absence of periportal thickening was recorded by examination of echo dense areas along the portal vein and its branches. Spleen length (SL), the presence of portal systemic collaterals and ascites were also recorded. The liver image pattern was assessed using the subcostal, transhepatic view and the substernal transverse view and sagital scans with children lying on their backs. The degree of periportal fibrosis (PPF) was described as image patterns (IP) using a grading system based on the appearance or texture of the liver parenchyma. Six image patterns were used ranging from A to F but scored as 0 to 8 as described in the Niamey protocol (Richter *et al*, 2000). Image patterns A and B were considered normal. Liver image pattern C was considered mild fibrosis, Liver image pattern D was considered moderate fibrosis, liver image pattern E and F were considered advanced periportal fibrosis. For urinary schistosomiasis, ultrasonography examination of the urinary tract (bladder, ureters and kidneys) were performed. The same equipment described above was used. All children were given about 400ml of water half an hour prior to examination in order to make sure their urinary bladders were full. Examination of the kidneys was performed using the left and right lateral views. Both kidneys and the proximal parts of the ureters were examined from lateral views using the mid axillary line. The urinary bladder shape and wall thickness was examined using the transverse view at the cross sectional diameter of the bladder while the child is lying in the supine position. This view was also used to visualize the distal part of the ureters. Pathological changes recorded in the kidneys included dilation of the renal pelvis and were defined as no dilation (fissure $\leq$ 1cm), moderately dilated (fissure > 1cm, parenchyma thickness >1cm) and marked hydronephrosis (parenchyma compressed, thickness < 1cm). Pathological changes of the ureters were recorded as absent if the ureters were not visualized, present (dilated) if the ureters were visualised at the proximal and/or distal 1/3 and present (grossly dilated) if the entire ureter was visualized. The bladder shape was defined as normal if it was rectangular or abnormal or distorted if it was round. The bladder wall irregularity was assessed and described as absent (if inner surface thickness < 5mm) or present (if inner surface thickness $\geq$ 5mm). Presence of multifocal lesions of the bladder wall was described as present if two or more lesions separated by a normal wall were observed. Thickening of the bladder wall was also assessed and described as absent (wall thickness < 5mm) or present (wall thickness $\geq$ 5mm). Presence of localized thickening of the bladder wall protruding into the lumen was also assessed and described as masses. A score of 2 was given for one mass. If more than one masses were observed, the total number of masses was calculated by adding 2 to the number of masses observed e.g. if 3 masses were observed, the total number of masses was calculated as 2 + 3 = 5). Presence of outgrowths of

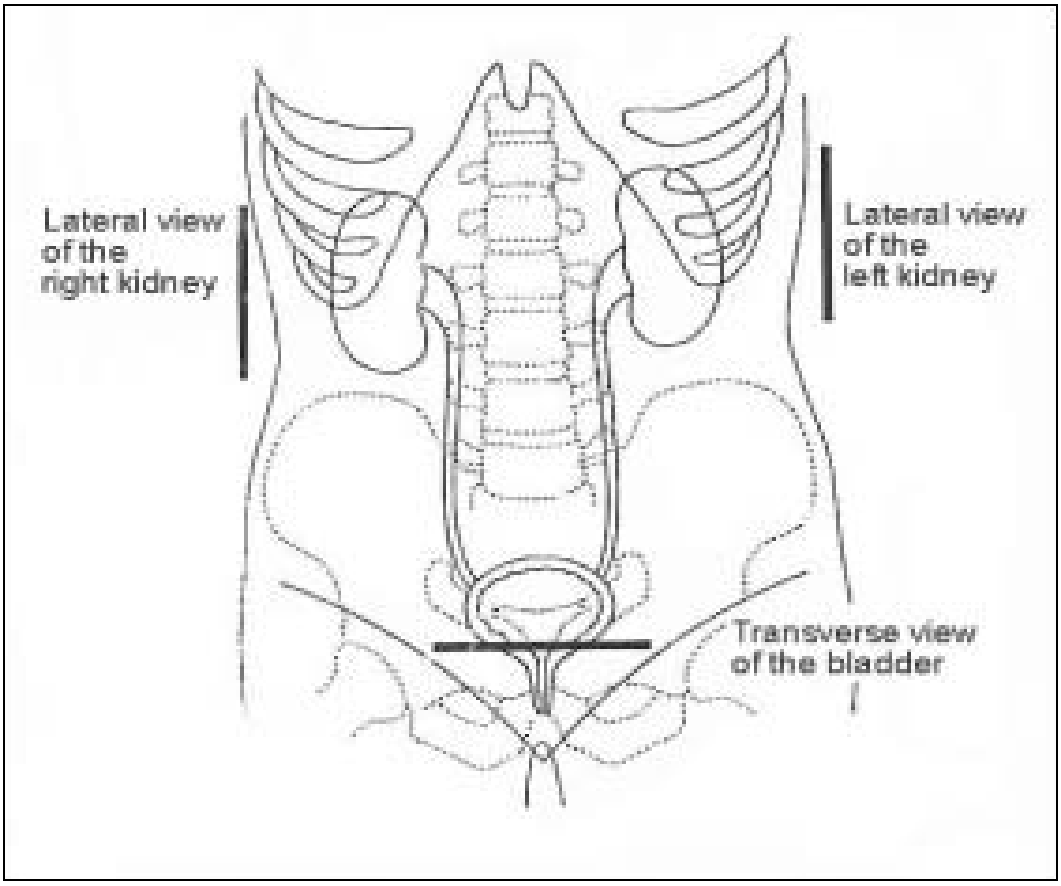
the bladder wall attached by a slender base was also assessed and described as pseudopolyps. Pseudopolyps were scored in the same way as for masses.

Figure 2.4 Standard scans for liver examination of *S. mansoni* related pathology (Adapted from Richter *et al*, 2000)



1. Longitudinal liver scans
   - 1a Left parasternal longitudinal view
   - 1b Right mid-clavicular view
   - 1c Right anterior axillary view
2. Substernal transverse view
3. Subcostal transhepatic view
4. Right oblique view
5. Left intercostal oblique view

Figure 2.5 Standard scans for examination of *S. haematobium* related pathology (Adapted from Richter *et al*, 2000).



2.5.3. Collection and examination of stool, urine and blood samples

Children were provided with plastic containers and requested to bring stool and urine samples on two consecutive days. Plastic containers with samples collected from each child were labelled with the child's identification number. Stool samples were examined for *S. mansoni* using the Kato Katz technique (WHO, 1994). Duplicate smears (41.7mg) were prepared from each stool sample. Intensity of infection for *S. mansoni* was expressed as the mean eggs/gram of faeces (epg) of the two samples (four smears). *S. mansoni* infection was classified as light (epg < 100), moderate (epg 100 – 399) and heavy (epg ≥ 400).

Urine samples were examined for *S. haematobium* eggs in 10ml urine according to the Nuclepore® filtration method (WHO, 1991). Egg counts were expressed as mean eggs/10ml of urine of the two samples. *S. haematobium* infection was classified as light (< 50 eggs/10ml of urine) and heavy (≥ 50 eggs/10ml of urine). Blood samples (approximately 3ml) were collected using plain vacutainer tubes or disposable syringes. Thick blood smears were prepared, stained with Giemsa and examined microscopically for malaria parasites. Malaria parasite density was estimated by counting the number of parasites per 200 leucocytes in thick films at 100x magnification. The number of malaria parasites per µL of blood was calculated assuming 8000 white blood cells/µL of blood. The number of malaria parasites counted per 200 WBC was transformed into malaria parasite density by multiplying by a factor of 40. Malaria parasite density was classified as low (< 5000 parasites/µL of blood) and high (≥ 5000 parasites/µL of blood. A blood slide was considered negative if no parasites

were observed after counting 100 fields. For quality control check a 10% random sample of blood slides, urine filters and Kato smears were examined by an independent technician.

2.5.4. Assessment for Hb concentrations

Hb concentrations were determined using a portable digital haemocue machine (HemoCue® Angelholm, Sweden). Anaemia was defined as Hb concentrations <120g/L and severe anaemia was defined as haemoglobin concentrations <80g/L based on normal range of Hb concentrations for school age children in Africa (WHO, 1996; Fleming and Menendez, 2004).

2.5.5. *P. falciparum* specific ELISA

2.5.5.1. Preparation of *P. falciparum* schizont antigens (PfSE)

P. falciparum schizonts (laboratory strain FCR3) were kindly provided by the laboratory of Professor Thor Theander at the Centre for Medical Parasitology, Faculty of Health Sciences of the University of Copenhagen, Denmark. The PfSE was chosen because it is among major antigens produced by asexual forms of *P. falciparum* that are known to elicit strong protective humoral immune resposes in natural *P. falciparum* infections and can be easily measured in immunological studies (Kilejian, 1980; Holder *et al* 1982; Migot-Nabias *et al*, 1999). The humoral immune response to PfSE is a reflection of the overall acquired immune response to the many malaria antigens to which the human host is exposed over a given period of time (Holder *et al* 1982; Migot-Nabias *et al*, 1999). The number of schizonts was determined using the haemocytometer method and the re-suspension adjusted to give a schizont count of 10^8 schizonts/µl. The cells were lysed by freeze-thaw by plunging into a water bath at 35^0C for about 10 minutes followed by freezing at -80^0C. The process was repeated two times. The lysed cells were stored at -80^0C. The schizonts were homogenised by ultrasound sonication in ice and the protein concentration of the antigen homogenate was measured using the BIORAD protein assay method as above. The final *P. falciparum* schizont antigen (PfSE) extract was transferred into vials and stored at -80^0C until needed for use.

2.5.5.2. Determination of specific antibodies against *P. falciparum*

Serum samples were obtained from blood samples collected from each child. Immunoglobulin G3 (IgG3) against *P. falciparum* schizont antigen (PfSE) were determined using the Enzyme Linked Immunosorbent Assay (ELISA) method. Serum samples were diluted in PBS with 0.1% Marvel (Skimmed milk powder, ISIS ApS, Århus V, Denmark) to 1:200 and incubated overnight at 4^0C in PfSE coated microplates (Greiner, Highbinding plates No. 65 5061). Coating was done using PfSE (8µg/ml) in coating buffer at a dilution of 1:130. Coated ELISA plates were incubated overnight at 4^0C. Washing was done using washing buffer (PBS with 0.03% Tween 20). After washing, the plates were blocked using blocking buffer (PBS with 0.05% Tween 20 and 1% Marvel) and incubated for one hour at room temperature. After washing, biotinylated mouse anti-human monoclonal antibody (IgG3) (Sigma B3523) was added as conjugates and incubated at room temperature for one hour. After washing, Streptavidin-peroxydase (Sigma No. S2438) was added and incubated again for one hour at room temperature. 1, 2-phenylenediamine dihydrochloride (OPD) tablets 2mg (DAKO No. S2045) was added (4 tablets per 12,000µl of distilled water) as substrate and incubated for 16 minutes. The reaction was stopped using sulphuric acid (0.5 MH_2S0_4) solution. The specific antibody response for PfSE was read at dual wavelength of 490/595 and expressed as optical density (OD) of the serum samples.

2.6. Outcome variables

The following outcome variables for malaria infection and anaemia, schistosome and STH infections and *P. falciparum* specific antibody responses were recorded during the baseline and follow up surveys.

2.6.1. Outcome variables for malaria and malaria/schistosome related morbidity

- Prevalence of malaria parasitaemia
- Malaria parasite density
- Clinical malaria attacks
- Prevalence of anaemia (Hb concentrations < 120g/L)
- Prevalence and degree of periportal fibrosis (PPF) expressed as liver image pattern scores
- Prevalence of enlarged liver and/or spleen determined by physical palpation and ultrasound examination
- Prevalence of enlarged portal vein diameter determined by ultrasound examination
- Prevalence of bladder or kidney pathology determined by ultrasound examination

2.6.2. Outcome variables for schistosome and intestinal helminth infections

- Prevalence of schistosome and STH infections
- Infection intensity of schistosome and STH infections

2.6.3. Outcome variables for *P. falciparum* specific antibody responses

- Seroprevalence of *P. falciparum* specific antibodies (IgG_3) in serum samples
- Levels of *P. falciparum* specific antibodies (IgG_3) in serum samples

2.7. Follow up surveys

The first (12 months) follow up survey was conducted between October to November 2007 and involved 655 children or 85.6% of children who were randomized into the intervention and control groups at the beginning of the antihelmintic intervention. The second (24 months) follow up survey was conducted between October to November 2008 and it involved 592 children or 89.9% children who were examined during the first follow up survey. During each follow up survey, stool, urine and blood samples were collected and examined to determine prevalence and intensity of malaria, schistosome and STH infections and the prevalence of anaemia in the same way as during the baseline survey. Clinical and ultrasound examination as well as *S. mansoni* and *P. falciparum* specific antibodies were also determined.

2.8. Data management and analysis

All data on clinical, ultrasound and laboratory examinations were recorded on data collection forms. After checking for quality and completeness, data were double entered in Dbase IV (Borland International, Scotts Valley, California) software. After cleaning, data was exported to STATA version 10 (STATA Cooperation, Texas, USA) for analysis. Children were divided into three age groups (3 – 5 years, 6 – 8 years and 9 – 13 years). Comparison of proportions was performed using the chi-square test. Comparison of geometric means of parasite counts (positive samples only) and haemoglobin (Hb) concentrations was performed using the student's t-test. In cells with less than five observations the Fisher's exact test was used instead of the t-test. Where more than two groups

were involved, comparison of means was performed using one way analysis of variance (ANOVA). Multiple logistic regression analysis was performed to determine relationships where binary outcome variables and categorical predictor variables were involved. Linear and multiple regression analysis were performed to determine relationships where continuous outcome and predictor variables were involved. All graphs were drawn using MS-Excel and STATA version 10 as appropriate. A p-value of < 0.05 was considered statistically significant.

2.9. References

Fleming, A. F. and Menendez, C. (2004). Anaemia in Africa: Normal range of haemoglobin concentrations in different age groups. In: Eldryd Parry., Richard Godfrey., David Mabey and Geofrey Gill (2004). Principals of Medicine in Africa. 3rd Edition. Cambridge University Press. London: UK. pp 924.

Holder, A. A and Freeman, R. R. (1982). Biosynthesis and processing of a P. falciparum schizont antigens recognised by immune serum and monoclonal antibody, *J. Exp. Med*, **156**: 1528-1538.

Kilejian, A. (1980). Stage-specific proteins and glycoproteins of Plasmodium falciparum: Identification of antigens unique to schizonts and merozoites. Proc. Natl. Acad. Sci., **77** (6): 3695-3699.

Kirkwood, B. R and Sterne, J. A. C. (2003): Sample Size Calculation. In: *Essential Medical Statistics*. Kirkwood, B & Sterne, J. A (editors), 2nd edition. Massachusetts: Blackwell Science Ltd, pp-413-422.

Massaga, J. J (2003). Malaria epidemiology in Tanzania. Paper presented to the workshop on Development of an Implementation Plan for Detection and Monitoring for Insecticide Resistance among Malaria Vectors in the Context of Insecticide Treated Bednets Scaling up in Tanzania. 24-26th November 2003, Hotel Kola Prieto, Tanga, Tanzania.

Migot-Nabis, F., Lute, E., A. J. F., Ringwald, P., Vailant, M., Dubois, B., Renault, A., Mayombo, J., Minh, T. N., Fievet, J., Mbessi, J. R., Millet, P., Deloron, P. (1999). Immune response against Plasmodium falciparum asexual blood stages in Garbonesse and Cameroonian children. *Am. J. Trop. Med. Hyg.,* **61** (3): 488-494.

Richter, J., Hatz, C., Campagne, G., Berquist, N., Jenkins, J (2000). Ultrasound in schistosomiasis: A Practical Guide to the Standardized use of Ultrasonography for the Assessment of Schistosomiasis Related Morbidity. World Health Organization. WHO/TDR/STR/SCH/001, Geneva. www.who.int/tdr.

Vennervald, J. B., Kenty, L., Butterworth, A. E., Kariuki, C. H., Kadzo, H., Ireri, E., Amaganga, C., Kimani, G., Mwatha, J., Otedo, A., Booth, M., Ouma, J. H., Dunne, D. W (2004). Detailed clinical and ultrasound examination of children and adolescents in Schistosoma mansoni endemic areas in Kenya: Hepatosplenic disease in the absence of portal fibrosis. *Trop Med Intel Health*, **9** (4): 1-10.

WHO (1991). Basic laboratory methods in medical parasitology. WHO, Geneva, Italy.

WHO (1994). Bench aids for the diagnosis of intestinal parasites. WHO, Geneva, Italy.

WHO (1996). Report of an informal consultation on hookworm infections and anaemia. WHO, Geneva: Mimeographed document, WHO/CTD/SIT/96.1

Chapter 3: Epidemiology of malaria and helminth co-infections in school and preschool children in Magu district, North-western Tanzania

Abstract

A study was undertaken to investigate the epidemiology of malaria and helminths co-infections and the prevalence of anaemia among school and pre-school children in Magu district, North-western Tanzania. Stool samples were collected and examined using the Kato Katz method to identify and quantify *S. mansoni and* Soil Transmitted Helminthiasis (STH) eggs. Urine samples were collected and examined using the urine filtration method to identify and quantify *S. haematobium* eggs. Giemsa stained blood smears were prepared and examined for malaria parasitaemia and Hb concentrations. Out of the 1546 children included in the analysis, 1079 (69.8%) were infected with one or more parasites. The prevalence of the parasite infections were: *P. falciparum* (29.8%), *S. mansoni* (39.7%), *S. haematobium* (19.7%), hookworms (15.9%) and *T. trichiura* (0.2%). Malaria-helminth co-infections were observed in 276 children or 60% of all children with *P. falciparum* infection. The prevalence and infection intensity of *S. mansoni*, *S. haematobium* and hookworms differed significantly across age groups as well as between schools with older children having higher prevalence and infection intensity compared to young children. For *P. falciparum* infection, the prevalence increased with age while malaria parasite density decreased with age. The prevalence of malaria parasitaemia was significantly associated with hookworm infection ($\chi^2 = 3.98$, $p = 0.046$). Malaria parasite density tended to decrease with increasing infection intensity of *S. mansoni* and *S. haematoium* with the difference being significant for *S. mansoni* infection only ($F = 6.9$, $p < 0.01$). Likewise, malaria parasite densities decreased significantly with increasing number of co-infecting helminth species ($F = 4.0$, $p < 0.01$). In the multivariate analysis, only *S. mansoni* infection was a significant predictor of malaria parasite density. The prevalence of anaemia was (34.4%) but without significant differences between boys and girls ($\chi^2 = 3.24$, $p = 0.072$). The highest prevalence of anaemia occurred in children co-infected with *P. falciparum*, *S. mansoni* and *S. haematobium*. Anaemia was significantly more prevalent in children with multiple parasite infections (two parasites or more) compared to children infected with single or no parasite infection ($\chi^2 = 21.60$, $p < 0.001$). Boys had significantly lower mean Hb concentrations compared to girls ($t = -2.08$, $p = 0.038$). Children with multiple parasite infections had significantly lower mean Hb concentrations compared to children infected with single or no parasite infection ($F = 7.3$, $p < 0.001$). Anaemia was significantly associated with malaria infection ($\chi^2 = 16.58$, $p < 0.001$) and with *S. haematobium* infection ($\chi^2 = 16.34$, $p < 0.001$). In the multivariate analysis, age group and hookworm infection were significant predictors of malaria infection. Likewise, *P. falciparum* and *S. haematobium* infections were significant predictors of anaemia. Findings of this study suggest that polyparasitism is common in school and pre-school children in Magu district with the most important parasite combinations involving *S. mansoni, P. falciparum* and *S. haematobium*. The results also suggest that hookworm infection tends to increase susceptibility to *P. falciparum* infection while concurrent *P. falciparum*, *S. mansoni* and *S. haematobium* infections tends to enhance the risk of lower Hb levels and anaemia.

3.1. Introduction

Malaria, schistosomiasis and soil transmitted helminth infections (STH) are the most important parasitic infections in Sub-Saharan Africa, where a significant proportion of the populations including school children are exposed to these infections. (Brooker *et al*, 2007; De Silva *et al*, 2003; Snow *et al*, 2005; Partney and Andrew, 1998; Mwangi *et al*, 2006). In Tanzania, these infections are a public health problem among school and pre-school children (Lwambo *et al*, 1999; Magnussen *et al*, 2001; Magnussen *et al*, 2002). Malaria, caused mainly by *P. falciparum* occurs throughout the country with varying levels of endemicity. Stable and perennial transmission occurs in the warm humid coastal regions and around the great Lakes (Mboera and Kitua, 2001; Mboera, 2004). Schistosomiasis and STH infections also occur throughout Tanzania. In the Lake Victoria basin, prevalence exceeding 50% has been reported (Archie *et al,* 2006; Lwambo *et al*, 1999). As a result of geographical overlap, malaria, schistosomiasis and the major STH (Hookworm, *Trichuris trichiura* and *Ascaris lumbricoides*) share not only the areas in which they occur, but also the human hosts (Partney and Andrews, 1998; Mwangi *et al*, 2006; Brooker *et al*, 2007). Children co-infected with these parasites develop less than optimal, have reduced learning and school achievements and have increased susceptibility to other infections (Grantham-McGregor *et al*, 2001; Brooker *et al* 2007; Hotez *et al,* 2004; Friedman *et al*, 2005; Ezeamama *et al*, 2005). Epidemiological studies have indicated that individuals co-infected with more than one parasite species are at risk of increased morbidity and mortality (Booth *et al*, 2004; Brooker *et al*, 2007; Egwunyenga *et al*, 2001; Howard *et al*, 2001; Tchuem Tchuente *et al*, 2003; Tshikuka *et al* 1996), as well as at a risk of developing frequent and more severe disease due to interactions among the infecting parasite species (Tshikuka *et al*, 1996; Nacher *et al* 2002; Sokhna *et al*, 2004). Helminth infected individuals are more likely to develop clinical *P. falciparum* malaria than helminth free individuals (Nacher *et al*, 2002; Spiegel *et al*, 2003). Concurrent parasitic infections also jointly contribute to anaemia. Hookworm and *T. trichiura* infections are associated with anaemia due to blood and iron loss into the intestinal tract while *S. mansoni* and *S. haematobium* infections cause blood loss in faeces and urine, respectively (Friedman *et al*, 2005; Hotez *et al*, 2004; Brooker *et al*, 2007). Considering the limited number of studies on interactions between malaria and helminth co-infections in human populations, the present study was undertaken to investigate the epidemiology of malaria and helminth co-infections and the prevalence of anaemia among school and pre-school children in the study area.

3.2. Methodology

3.2.1. Study area and population

The study was conducted in Magu district, North-Western Tanzania. The study took place between October to November, 2006 and involved six selected primary schools. School and pre-school children aged 3 – 13 years were included in the study. More details about the study area and population, study design and selection of schools and children is explained in details in chapter 2.

3.2.2. Collection and examination of stool, urine and blood samples

Children were provided with plastic containers and requested to bring stool and urine samples on two consecutive days at about 10:00 am in the morning. Stool samples were examined for *S. mansoni* and intestinal helminths (*T. trichiura, A. lumbricoides* and hookworm) using the Kato Katz technique (WHO, 1994). Duplicate smears (41.7mg) were prepared from each stool sample. Intensity of infection for *S. mansoni* and intestinal helminths were expressed as the mean eggs per

gram of faeces (epg) of the two samples (four smears). Urine samples were examined for *S. haematobium* eggs in 10ml urine according to the nuclepore filtration method (WHO, 1991). Blood samples (approximately 3ml) were collected using plain vacutainer tubes or disposable syringes. Thick blood smears were prepared, stained with Giemsa and examined microscopically for malaria parasites. Hb concentrations were determined using a portable HaemoCue photometer. Anaemia was defined as Hb < 120g/L and Hb < 80g/L as severe anaemia. Quality control was performed by re-examining 10% randomly selected blood slides, urine filters and Kato smears by an experienced independent technician.

3.2.3. Data analysis

Data were double entered into Dbase V software and analyzed using STATA Version 10. Parasite counts were normalized by log transformation. Infection intensities were calculated as geometric mean of eggs per gram of faeces for *S. mansoni* and hookworm infections, eggs per 10ml of urine for *S. haematobium* and parasites per microlitre of blood for *P. falciparum* based on positive samples only. The student's t-test and one way analysis of variance (ANOVA) was used to compare geometric mean parasite counts and mean Hb concentrations where two or more than two groups were compared, respectively. For parasite counts, the t-test and ANOVA were performed on log transformed data of positive samples only whereas for Hb concentrations the t-test and ANOVA were performed for all samples examined on original sacle. Geometric mean parasite counts and their corresponding 95% confidence intervals (95% CI) were calculated using STATA version 10 for positive samples only. The Chi-square test was used to compare proportions and to test for association between malaria prevalence, anaemia prevalence and prevalence of helminth infections between exposure groups. Multivariate regression analysis was used to determine significant predictors of malaria infection, prevalence of anaemia and malaria parasite densities. Graphs were drawn using STATA 10 or MS-Excel as appropriate. Tests were considered statistically significant at $p < 0.05$.

3.3. Results

A total of 1615 school and pre-school children were examined. Pre-school children were 372 or 23% of all children examined. Children where complete information was available were included in the analysis (1546) of whom 759 (49.1%) were boys. Overall mean age was 7 years. Table 3.1 shows the demographic profile of the studied population.

Table 3.1 Demographic profile of the study population by school, sex and age groups (n = 1546).

School/Sex	Age group and no. examined (%)				P-Value
	3 – 5 (n = 333)	6 – 8 (n = 975)	9 – 13 (n = 238)	Total (%)	
Mwamayombo (n = 279)					
Boys	39 (45.9)	95 (54.0)	5 (27.8)	139 (49.8)	
Girls	46 (54.1)	81 (46.0)	13 (72.2)	140 (50.2)	0.947
Nyashimo (n = 302)					
Boys	17 (43.6)	109 (47.2)	12 (37.5)	138 (45.7)	
Girls	22 (56.4)	122 (52.8)	20 (62.5)	164 (54.3)	0.137
Bulima (n = 211)					
Boys	17 (44.7)	50 (45.1)	34 (54.8)	101 (47.9)	
Girls	21 (55.3)	61 (54.9)	28 (45.2)	110 (52.1)	0.542
Milambi (n = 202)					
Boys	27 (54.0)	60 (48.8)	16 (55.2)	103 (51.0)	
Girls	23 (46.0)	63 (51.2)	13 (44.8)	99 (49.0)	0.776
Ihale (n = 255)					
Boys	25 (50.0)	68 (43.9)	27 (54.0)	120 (47.0)	
Girls	25 (50.0)	87 (56.1)	23 (46.0)	135 (53.0)	0.339
Ijitu (n = 297)					
Boys	38 (53.5)	92 (51.4)	28 (59.6)	158 (53.2)	0.271
Girls	33 (46.5)	87 (48.6)	19 (40.4)	139 (46.8)	
Total					
Boys	163 (49.0)	474 (48.6)	122 (51.3)	759 (49.1)	
Girls	170 (51.0)	501 (51.4)	116 (48.7)	787 (50.9)	0.479

3.3.1. Prevalence and infection intensities of parasitic infections

Out of the 1546 children included in the analysis, 1079 (69.8%) were infected with at least one of the parasites *P. falciparum, S. mansoni, S. haematobium,* hookworm and *T. trichiura. S. mansoni* infections were generally light to moderate with only 59 children (9.6%) being heavily infected (epg $\geq$ 400). Whereas 94 children (3.8%) had heavy *S. haematobium* infections ($\geq$ 50 eggs/10ml of urine), all hookworm infections were light (epg < 2000). Only three children (0.2%) were infected with *T. Trichiura.* Due to this low prevalence of infection, *T. trichiura* was excluded in further analysis. The prevalence of individual parasite species and the respective infection intensities are shown in table 3.2.

Table 3.2 Prevalence (n = 1546) and infection intensities (expressed as geometric mean parasite count of positive samples only) of parasitic infections by sex and age groups in school and pre-school children in Magu district, Tanzania.

Infection/Sex	**Age group and no. infected (%)**				**Geometric mean parasite count (95% CI)**			
	3-5	6-8	9-13	Total	3-5	6-8	9-13	Total
P. falciparum								
Boys	48 (41.7)	149 (31.4)	45 (36.9)	242 (31.9)	838 (565-1243)	626 (502-780)	483 (316-737)	632 (531-752)
Girls	44 (25.9)	136 (27.2)	38 (32.8)	218 (27.7)	603 (377-964)	730 (586-910)	359 (247-533)	621 (519-742)
Total	92 (27.6)	285 (29.2)	83 (34.9)	460 (29.8)	715 (530-967)	674 (577-787)	422 (318-560)	627 (553-710)
P-value	0.467	0.141	0.504	0.072	0.281	0.331	0.298	0.881
S. mansoni								
Boys	39 (23.9)	203 (42.8)	67 (54.9)	309 (40.7)	45 (26-76)	49 (40-61)	101 (71-145)	49 (48-68)
Girls	36 (21.2)	206 (41.1)	62 (53.5)	304 (38.6)	59 (36-96)	43 (35-52)	45 (32-64)	45 (38-53)
Total	75 (22.5)	409 (42)	129 (54.2)	613 (39.7)	51 (36-73)	46 (40-53)	68 (53-89)	51 (45-57)
P-value	0.548	0.589	0.820	0.402	0.478	0.321	0.001	0.044
S. haematobium								
Boys	26 (16.0)	99 (20.9)	40 (32.9)	165 (21.7)	31 (14-65)	17 (12-24)	14 (8-28)	18 (14-24)
Girls	17 (10.0)	98 (19.6)	25 (21.6)	140 (17.9)	10 (4-26)	16 (11-22)	11 (5-23)	14 (10-18)
Total	43 (12.9)	197 (20.2)	65 (27.3)	305 (19.7)	20 (11-36)	17 (13-20)	13 (8-21)	16 (13-20)
P-value	0.105	0.596	0.052	0.051	0.075	0.710	0.496	0.190
Hookworm								
Boys	11 (6.7)	80 (16.9)	25 (20.5)	116 (15.3)	42 (20-92)	57 (43-77)	81 (48-135)	60 (47-76)
Girls	26 (15.3)	74 (14.7)	29 (25.0)	129 (16.4)	67 (40-112)	44 (33-59)	47 (28-81)	49 (39-61)
Total	37 (11.1)	154 (15.8)	54 (22.7)	245 (15.9)	59 (39-88)	51 (41-62)	60 (42-87)	54 (46-63)
P-value	0.013	0.367	0.492	0.551	0.299	0.210	0.153	0.216

The prevalence of *S. mansoni, S. haematobium* and hookworm infections differed significantly across age groups ($p > 0.001$) whereby older children (6 – 8 years and 9 – 13 years) had higher prevalence of infection compared to younger children (3 – 5 years). Likewise, the infection intensity of *S. mansoni, S. haematobium* and hookworm differed significantly across age groups ($p > 0.001$) whereby children in higher age groups had higher parasite loads compared to children in the lower age group. For *P. falciparum* infection, an inverse pattern was observed whereby children in the lower age group had higher parasite loads compared to children in the higher age groups (Table 3.2).
The prevalence and infection intensity for malaria, schistosoma and hookworm differed significantly between schools ($p<0.001$). The greatest variation in prevalence of infection was observed for *S. mansoni* whereby the overall prevalence was 39.7% (95% CI 37.2 – 42.1). The minimum and maximum levels were 23.9% and 59.2% at Ijitu and Bulima primary primary schools, respectively. Less variation occurred for the prevalence of *S. haematobium* infection (overall prevalence 19.7%, 95% CI 17.8 – 21.8) and hookworm infection (overall prevalence 15.9%, 95% CI 14.1 – 17.8). The variation in the intensity of infection followed a similar pattern whereby schools with the highest prevalence levels also had the highest levels of heavy infections.

3.3.2. Prevalence of single and multiple parasite infections

Out of the 1079 infected children, 430 (39.9%) harboured more than one parasite species. Table 3.3 shows the prevalence of single and multiple parasites infections observed.

Table 3.3 Prevalence of single and multiple parasite infections (by sex) in school and pre-school children in Magu district, Tanzania (n = 1546).

Infection status	Number (%)			P-Value
	Total	Boys	Girls	
Not infected	467 (30.2)	223 (29.4)	244 (31)	0.487
S. mansoni only	290 (18.8)	137 (18.1)	153 (19.4)	0.484
S. haematobium only	107 (6.9)	44 (5.8)	63 (8.0)	0.087
Hookworm only	67 (4.3)	25 (3.3)	42 (5.3)	0.049
P. falciparum only	184 (11.9)	93 (12.3)	91 (11.6)	0.675
S. Mansoni and S. haematobium	60 (3.9)	34 (4.5)	26 (3.3)	0.231
S. mansoni and hookworms	54 (3.5)	22 (2.9)	32 (4.1)	0.267
S. mansoni and *P. falciparum*	117 (7.6)	65 (8.5)	52 (6.6)	0.146
S. haematobium and *P. falciparum*	44 (2.9)	28 (3.7)	16 (2.0)	0.050
Hookworms and *P. falciparum*	32 (2.1)	16 (2.1)	16 (2.0)	0.918
S. mansoni, *S. haematobium* and *P. falciparum*	30 (1.9)	17 (2.2)	13 (1.7)	0.402
S. mansoni, *S. haematobium* and hookworms	20 (1.3)	15 (2.0)	5 (0.6)	0.020
Others	74 (4.9)	40 (5.3)	34 (4.3)	0.382
Total	**1546 (100)**	**759 (49.1)**	**787 (50.9)**	**0.479**

S. mansoni infections occurred as single as well as a multiple species infection in almost equal proportions (18.8% and 20.95, respectively). *P. falciparum, S. haematobium* and hookworms infections

occurred more frequently as multiple species infections than single species infections. Figures 3.1 and 3.2 summarize the prevalence of single and multiple parasite species infections.

Figure 3.1 Prevalence of single and multiple parasitic infections by sex (n = 1079, boys 536, girls 543)

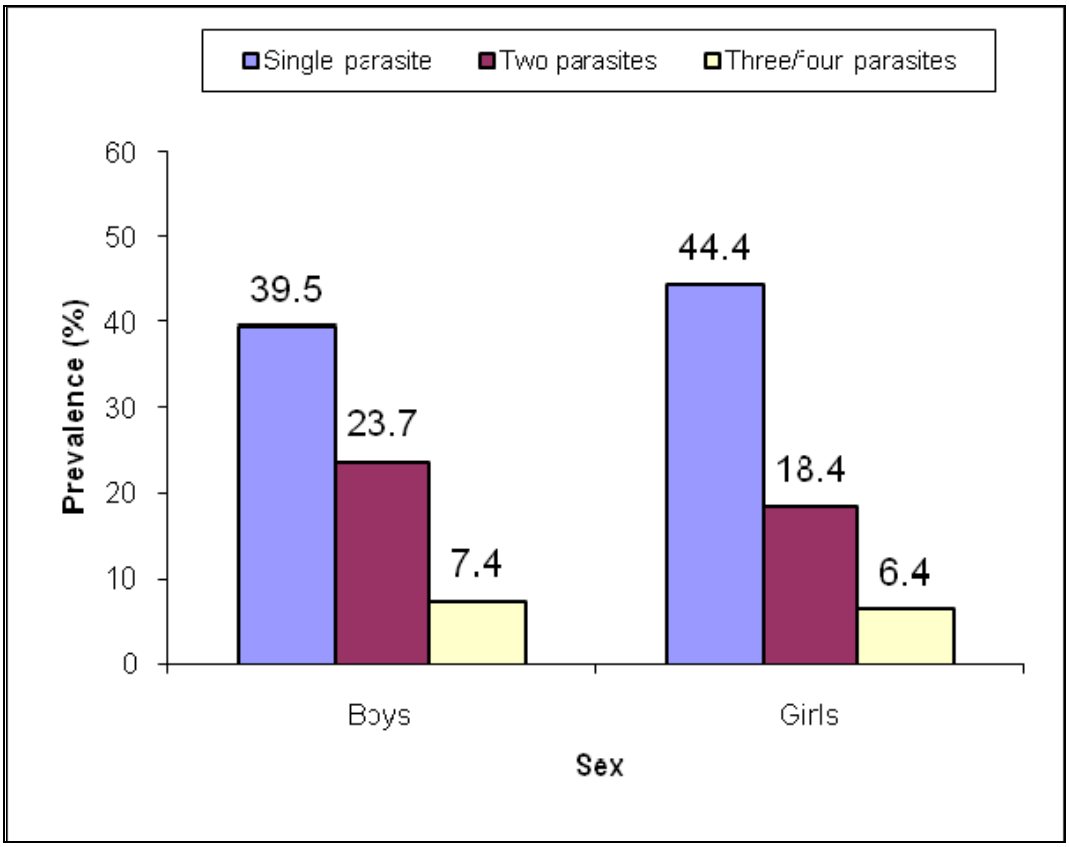


Figure 3.2 Prevalence of single and multiple parasitic infections by age groups (n = 1079).

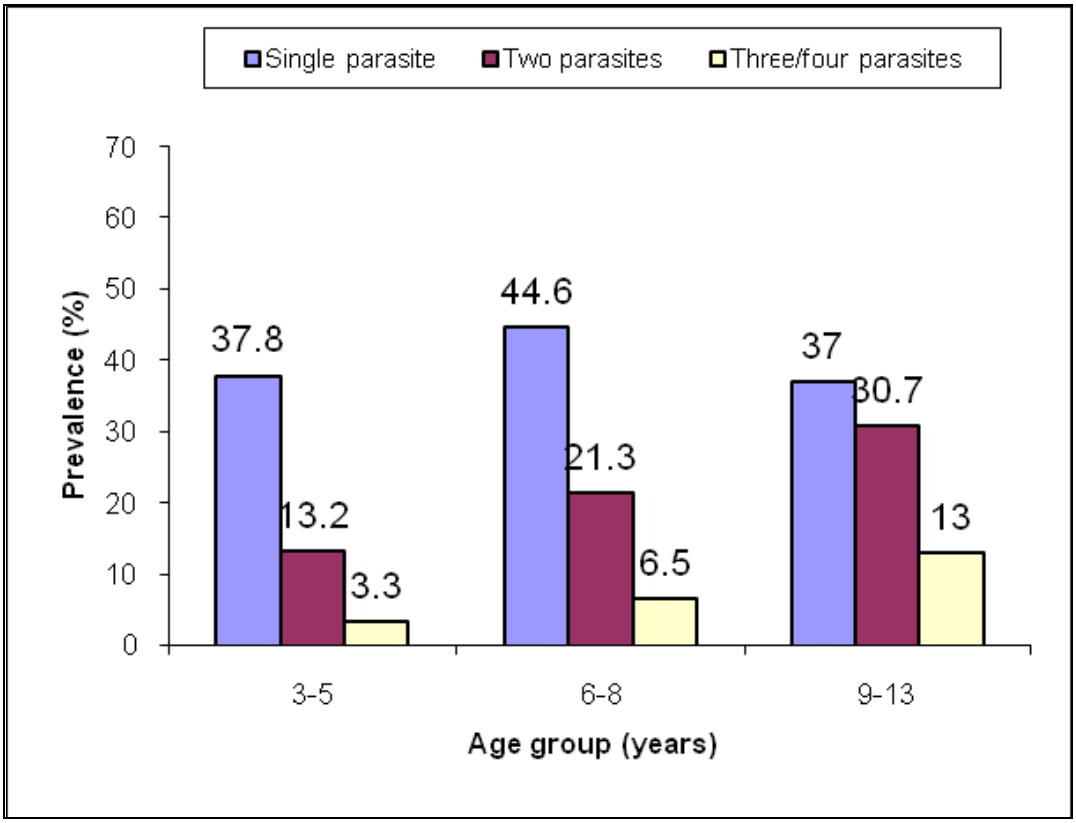


Figure 3.1 shows that multiple parasite infections occurred more frequently in boys than girls (χ^2 = 7.98, p < 0.01). Figure 3.2 shows that multiple parasite infections occured more frequently in older children (9 – 13 years) compared to younger children (3 -5 and 6 – 8 years) (χ^2 = 51.07, p < 0.001).

3.3.3. Prevalence of malaria parasitaemia and and association with helminth co-infections

Malaria and helminth co-infections were observed in 276 or 60% of all children who were positive for malaria parasites. The most common parasite co-infections were *P. falciparum* and *S. mansoni* (27.2%), *P. falciparum* and *S. haematobium* (10.2%), *P. falciparum* and hookworm (7.4%), *P. falciparum, S. mansoni* and *S. haematobium* (7%), *P.falciparum, S.mansoni* and hookworm (6.5%) and *P. falciparum*, *S. haematobium* and hookworm (3.0%). Malaria and helminth co-infections tended to occur more frequently in boys than girls though the difference was not significant (χ^2 = 3.21, p = 0.073). Further, malaria and helminth co-infections occurred more frequently in older children (9 – 13 years) compared to younger children (3 – 5 and 6 – 8 years) and the difference was significant (χ^2 = 19.34, p < 0.001). Malaria parasitaemia was significantly more prevalent in hookworm infected children than in hookworm free children (35.1% vs 28.8%) (χ^2 = 3.98, p = 0.046). The prevalence of

malaria parasitaemia tended to increase with increasing number of co-infecting helminth species. The prevalence of malaria parasiteamia was 29%, 35% and 41.2% in children harbouring one, two and three helminth specie, respectively, compared to 28.3% in helminth free children. However, the difference was not significant ($\chi^2 = 5.63$, $p = 0.131$).

Table 3.4 shows results of a multivariate logistic regression analysis with adjusted odds ratios and p-values. Independent variables included in the logistic regression model were sex, age group, *S. mansoni* infection, *S. haematobium* infection, hookworm infection and the presence of any helminth infection.

Table 3.4 Results of multivariate logistic regression analysis showing predictors of malaria infection (N = 1546).

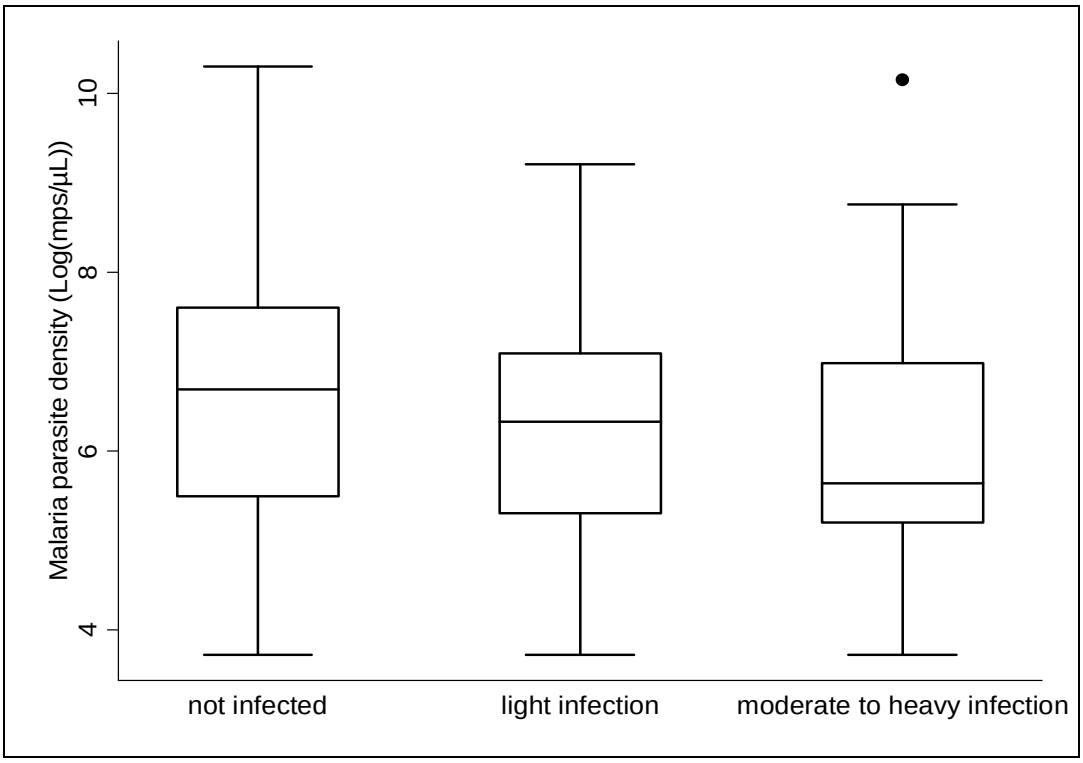
Independent variable	Categories	Adjusted OR (95% CI)	P-Value
Sex			
	Male	1	
	Female	0.814 (0.654 - 1.013)	0.066
Age group (years)			
	3 – 5	1	
	6 – 8	1.08 (0.816 - 1.423)	0.597
	9 - 13	1.442 (1.01 – 2.070)	0.047
Hookworm infection			
	Negative	1	
	Positive	1.352 (1.01 - 1.81)	0.043

Hookworm infection was a predictor of malaria infection in the final model. Age group was also a predictor of malaria infection whereby children in the age group of 9 – 13 years had an increased risk of malaria infection compared to children in the lower age groups (3 – 5 and 6 – 8 years). Presence of any helminth infection, *S. mansoni* and *S. haematobium* were not predictors of malaria infection

3.3.4. Malaria parasite density and association with helminth-coinfections

Except for hookworm infection, malaria parasite density was negatively correlated with helminth infections (prevalence and infection intensity). Figure 3.3 shows the relationship between malaria parasite density and *S. mansoni* infection while the relationship between malaria parasite density and the number of co-infecting helminth species is shown in figure 3.4.

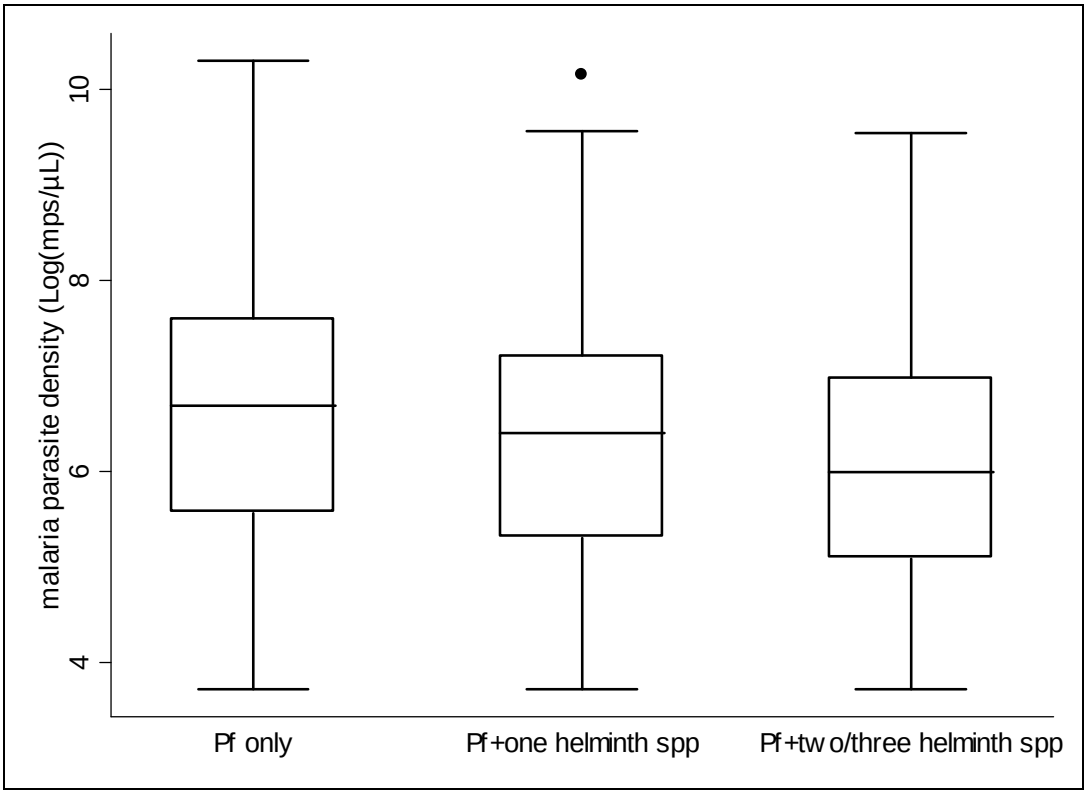
Figure 3.3 Box and whisker plot showing the relationship between median and range of log transformed values of malaria parasite density (Log(mps/µL)) and *S. mansoni* infection status



The thick line inside each box represents the median value. The lower and upper edge of each box indicates the 25th and 75th percentiles, respectively. The lower and upper whiskers represent the lower and upper values (range), respectively, excluding outliers. not infected (n = 933), light infection (n = 396) and moderate to heavy infection (n = 217).

Malaria parasite densities tended to decrease with increasing infection intensity of *S. mansoni*. Geometric mean malaria parasite density for children without *S. mansoni* infection was 745 (95% CI 633-879) and was significantly higher compared to 551 (95% CI 434-700) and 399 (95% CI 297-534) for children with light and moderate to heavy *S. mansoni* infection, respectively ($F = 6.9$, $p < 0.01$) (Fig. 3.3). Figures 3.4 shows the relationship between malaria parasite densities and overall helmnth co-infections. Malaria parasite densities tended to decrease with increasing number of co-infecting helminth species. Geometric mean malaria parasite density for children without any helminth infection (malaria only) (n = 184) was 779 (95% CI 640-948), significantly higher compared to 604 (95% CI 499-731) and 425 (95% CI 319-566) for children with one and two or more co-infecting helminth species, respectively ($F = 4.0$, $p < 0.01$).

Figure 3.4 Box and whisker plot showing the relationship between median and range of log transformed values of malaria parasite density (Log(mps/μL)) and co-infection status.



The thick line inside each box represents the median value. The lower and upper edge of each box indicates the 25th and 75th percentiles, respectively. The lower and upper whiskers represent the lower and upper values (range), respectively, excluding outliers.
Co-infection status: Pf only = *P. falciparum only (n =184)*; Pf+one helminth spp = *P. falciparum* plus one helminth species (n = 191); Pf+two/three helminth spp = *P. falciparum* plus two or three helminth species (*n = 85*)

Table 3.5 shows results of multiple linear regression analysis of malaria parasite density and log transformed helminth egg counts adjusted for age group and sex.

Table 3.5 Results of multiple linear regression analysis showing predictors of malaria parasite density after adjusting for age group and sex (n = 460).

Independent variable	**β (95% CI)**	**P – Value**
Age group	- 0.183 (- 0.385 – 0.020)	0.077
Sex	- 0.046 (- 0.291 – 0.199)	0.713
S. mansoni egg count (Log(epg))	- 0.091 (- 0.149 – - 0.034)	0.002
S. haematobium egg count (Log(eggs/10ml))	- 0.071 (- 0.159 – 0.017)	0.113

Table 3.5 shows that *S. mansoni* egg count was a significant negative predictor of malaria parasite density. *S. haematobium* egg count was also a negative predictor of malaria parasite density though the effect did not reach significant level.

3.3.5. Prevalence of anaemia and association with infection status

Out of the 1546 children, 532 (34.4%) were anaemic and only 16 (1.0%) were severely anaemic. Overall mean Hb concentration was 124 g/L (95% CI 123-125). The prevalence of anaemia and mean Hb levels are shown in table 3.6.

Table 3.6 Prevalence of anaemia (Hb < 120g/L) and mean haemoglobin levels by sex and age groups

Age group (years)	**Number with anaemia (%)**			**Mean Hb (g/L) (95% CI)**		
	Boys	Girls	Total	Boys	Girls	Total
3 – 5	58 (35.6)	55 (32.3)	113 (33.9)	123 (120-126)	124 (122-126)	124 (122-125)
6 – 8	171 (36.1)	166 (33.1)	337 (34.6)	123 (122-125)	125 (123-128)	124 (123-125)
9 – 13	49 (40.2)	33 (28.4)	82 (34.5)	122 (120-124)	126 (123-128)	124 (122-125)
Overall	278 (36.6)	254 (32.3)	532 (34.4)	123 (122-124)	124 (123-125)	124 (123-125)

Boys had significantly lower mean Hb concentrations (123, 95% CI 122-124) compared to girls (124, 95% CI 123-125) ($t = -2.08$, $p = 0.038$).

Table 3.7 Prevalence of anaemia and mean haemoglobin levels in relation to infection with single parasite species (n = 1546).

Infection status	**Number with anaemia (%)**	**Mean Hb (g/L) (95% CI)**
P. falciparum		
Not infected	340 (31.3)	124 (123-125)
Light infection	173 (40.9)	122 (121-124)
Heavy infection	19 (51.4)	115 (110-120)
P-Value	< 0.001	< 0.001
S. mansoni		
Not infected	315 (33.8)	124 (123-125)
Light infection	137 (34.6)	123 (122-125)
Moderate/heavy infection	8 (36.9)	122 (120-124)
P-Value	0.684	0.053
S. haematobium		
Not infected	397 (32.0)	124 (123-125)
Light infection	89 (42.2)	122 (120-124)
Heavy infection	46 (48.9)	120 (117-123)
P-value	< 0.001	<0.01
Hookworms		
Not infected	444 (34.0)	124 (123-125)
Light infection	90 (36.7)	124 (122-125)
P-Value	0.404	0.989

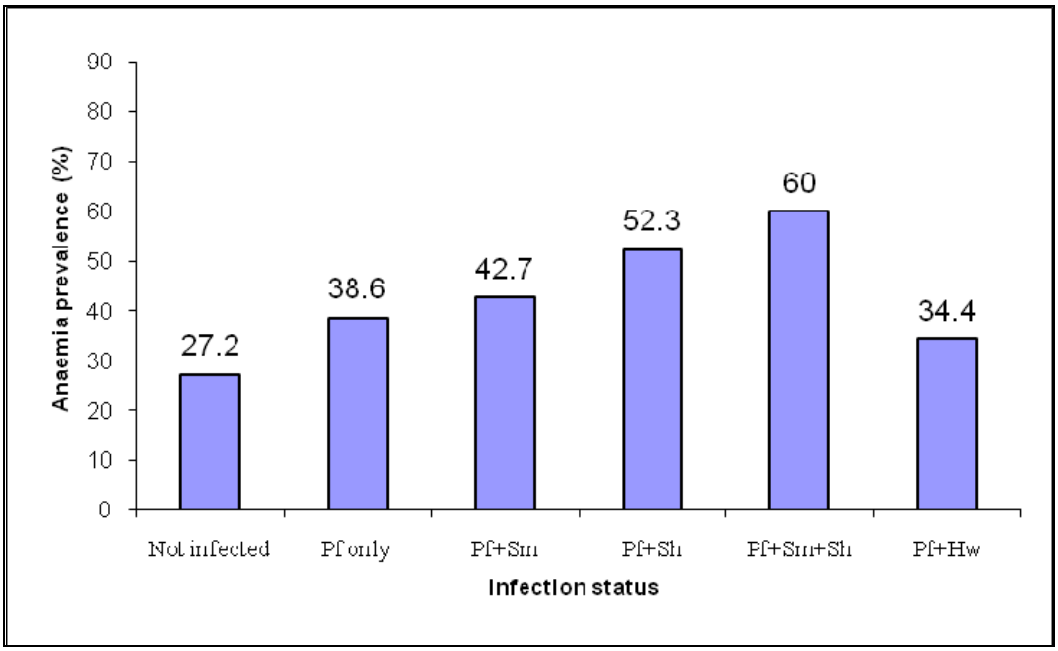
The prevalence of anaemia was significantly associated with malaria infection ($\chi^2 = 15.58$, $p < 0.001$) and *S. haematobium* infection ($\chi^2 = 16.34$, $p < 0.001$). For *P. falciparum* and *S. haematobium* infections, mean Hb levels decreased significantly with increasing infection intensities ($p < 0.01$). For *S. mansoni* infection, there was no significant decrease in Hb concentrations with increasing infection intensity ($p = 0.053$) (Table 3.7).

Table 3.8 Prevalence of anaemia and mean Hb levels in relation to infection with multiple parasite species (n = 1546).

Infection status	**Number with anaemia (%)**	**Mean Hb (95% CI)**
Not infected	127 (27.2)	126 (125-127)
Single parasite infection	225 (34.7)	123 (122-125)
Two parasite infection	134 (41.2)	121 (120-123)
Three/four parasite infection	46 (43.8)	122 (119-125)
P-value	< 0.001	< 0.001

Table 3.8 shows that anaemia was more prevalent in children with multiple parasite infections (two parasites or more) compared to children infected with single or no parasite infection. Likewise, children with multiple parasite infections had significantly lower mean haemoglobin concentrations compared to children infected with single or no parasite infection.

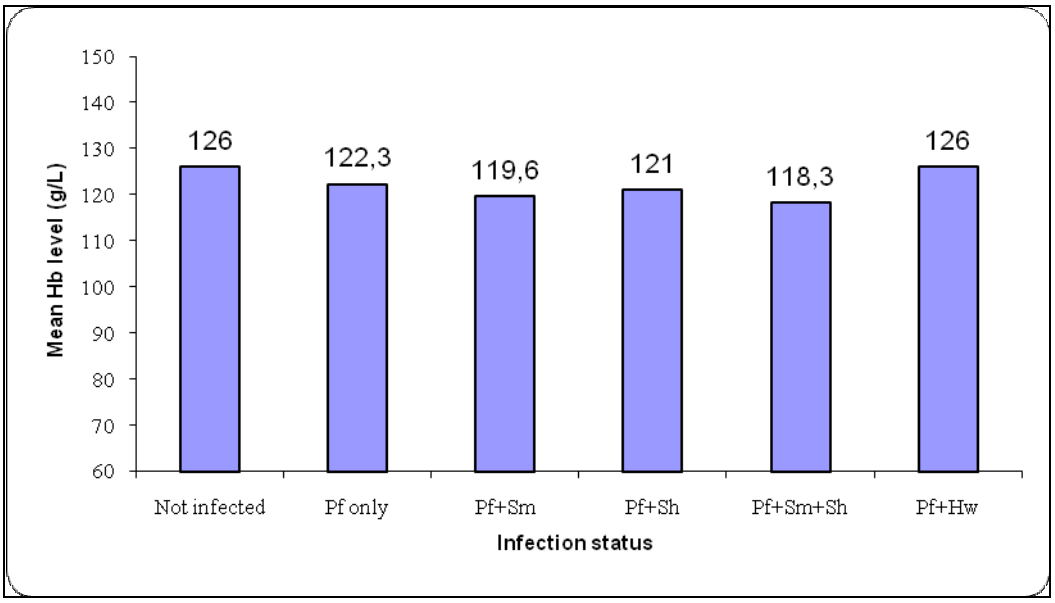
Figure 3.5 Prevalence of anaemia in relation to malaria and helminth co-infections.



Infection status: not infected (n =467) Pf = *P. falciparum only (n = 184)*; *Sm = S. mansoni (Pf+Sm n = 188)*; Sh = *S. haematobium(Pf+Sh n= 100) (Pf+Sm+Sh n = 43)*; Hw = Hookworms (*Pf + Hw n = 86)*

Overall, children who were not infected with any parasite had the lowest prevalence of anaemia (27.2%). Except for *P. falciparum* and hookworm co-infections, children who were infected with more than one parasite species tended to have higher prevalence of anaemia compared to children infected with one parasite. The highest prevalence of anaemia (60%) was observed in children co-infected with three parasites *P. falciparum*, *S. mansoni* and *S. haematobium* (Fig 3.5).

Figure 3.6 Mean haemoglobin levels in relation to malaria and helminth co-infections.



Infection status: Pf = *P. falciparum*; Sm = *S. mansoni*; Sh = *S. haematobium*; Hw = Hookworms: Not infected (*n = 467*), Pf only *(n = 184),* pf+Sm *(n = 188),* Pf+Sh *(n = 100),* Pf+Sm+Sh *(n = 43),* Pf+Hw *(n = 86).*

Overall, children who were not infected with any parasite had the highest mean Hb levels (125, 95% CI 124-1276). Except for *P. falciparum* and *hookworm* co-infections, children who were infected with more than one parasite species tended to have lower mean haemoglobin levels compared to children infected with one parasite. There was a significant difference in the prevalence of anaemia and mean haemoglobin levels between uninfected children and those infected with one or more parasites ($p < 0.01$) (Figure 3.5 and 3.6, respectively).

3.3.6. Predictors of anaemia

Multivariate logistic regression analysis was performed to identify predictors of anaemia. Variables included in the analysis were age group, sex, malaria infection, *S. mansoni* infection, *S. haematobium* infection, hookworm infection and the presence of any helminth infection. The results of the final model are summarised in table 3.10.

Table 3.9 Results of multivariate logistic regression analysis showing important predictors of anaemia with adjusted odds ratios and p-values (N = 1546).

Independent variable	Categories	Adjusted OR (95% CI)	P-Value
Sex			
	Male	1	
	Female	0.82 (0.670 - 1.017)	0.072
Malaria infection			
	Not infected	1	
	Light infection	1.51 (1.195 - 1.905)	< 0.01
	Heavy infection	2.25 (1.165 – 4.356)	0.016
S. haematobium infection	Not infected	1	
	Light infection	1.55 (1.146 – 2.090)	<0.01
	Heavy infection	2.01 (1.320 - 3.073)	<0.01

P. falciparum and *S. haematobium* infections were found to be significant predictors of anaemia after adjusting for age and sex (Table 3.9).

3.4. Discussion

Malaria, schistosomiasis and STH are a major pulic health problem particularly to school and pre-school children in SSA where their occurrence as multiple species infections is known to be the norm. Understanding the epidemiology of these infections among school and pre-school children and their contribution to ill-health is important as findings may support design of integrated disease control strategies. Results of this study demonstrate that malaria, schistosomiasis and soi;-transmitted helminth infections are prevalent in school and pre-school children in Magu district. The results represent one among very few community based studies that have investigated malaria and helminth co-infections in Tanzania and in the Lake Victoria basin in particular. Other studies in the Sub-Saharan Africa region also observed the occurrence of concurrent parasitic co-infections in humans (Brooker *et al*, 1999; Brooker *et al*, 2000; Howard *et al*, 2002; Jennifer Keizer, 2002; Tchuem Tchuente *et al*, 2003). The most prevalent parasite species in the studied population were *S. mansoni*, *P. falciparum* and *S. haematobium*. The major STH infections hookworm and *T. trichiura* were the least prevalent. *Ascaris lumbricoides* was not detected also in accordance with the study of Lwambo *et al* (1999) which found this specie to be rare. The observed prevalence of *S. mansoni* and *S. haematobium* are in accordance with previous studies in the area and is related to the occurrence of the snail intermediate hosts for *S. mansoni* and *S. haematobium* and their ecological preferences (Webbe *et al*, 1962; Lwambo *et al*, 1999; Ajanga *et al*, 2006). The low prevalence of STH infections in the studied population could be as a result of the relatively younger age of most of children examined as the prevalence of STH particularly hookworm peaks in early adulthood. Another possible reason could be the overall distribution of STH in East Africa. While hookworm infections are widely distributed in East Africa, *A. lumbricoides* and *T. trichiura* have limited distribution occurring more frequently in the Western part of Lake Victoria

and less frequently in the Southern and Eastern parts of the lake (Brooker *et al*, 2009). In this study, the two species of hookworm *N. americanus* and *A. duodenale* were not differentiated though previous evidence suggest that *N. americanus* is the predominant specie in East Africa (Stoltzfus *et al*, 1997). For schistosomiasis and hookworm infections, the observed prevalence and infection intensity were generally age and sex dependent which reflects the fact that infection levels are explained by water contact patterns, duration of exposure to infection and acquired immunity (Stoltzfus *et al*, 1997: Briand *et al*, 2005: Kabatereine *et al*, 2004, Hotez *et al*, 2004). The study also observed significant variation among schools of both prevalence and infection intensities of *S. mansoni* and *S. heamatobium* which could be explained by variations in exposure, focal nature of schistosomiasis and over-dispersed distribution of heavy and light infections between and within communities (Hotez *et al*, 2004). Malaria parasite densities decreased with increasing age which is a normal trend in malaria endemic areas and is related to development of anti-malarial specific immunity.

This study has demonstrated that malaria and helminth co-infections are very common in the study area and interactions exist among them. Majority of children who were infected with *P. falciparum* were concurrently infected with one or more helminth species. Hookworm infection was found to be positively associated with malaria infection and malaria parasite density, a finding which concurs with findings of Hillier *et al*, 2008; Nacher *et al*, 2002; Nkuo-Akenji *et al*, 2006 and Spiegel *et al*, 2003 but constrats findings of Shapiro *et al*, 2005. Although this relationship was of borderline significance, it may point out to important biological interactions between these infections. The study of Hillier *et al* (2008) in Uganda, which also observed an increased risk of *P. falciparum* infection among pregnant women infected with hookworm tends to strengthen the evidence of a true biological association between these two parasitic infections. However, this association needs to be confirrmed by further longitudinal studies. The mechanisms underlying the observed association between *P. falciparum* and hookworm infections are not clearly understood. There are suggestions that environmental, socio-economic and behavioural factors could act as shared risk factors for exposure to both infections (Mwangi *et al*, 2006; Hillier *et al*, 2008). Other authors suggest the involvement of immunological mechanisms which may lead to increased susceptibility of helminth infected individuals to *P. falciparum* infection (Maizels *et al*, 2004; Spiegel *et al*, 2003; Sokhna *et al*, 2004). Except for hookworm infection which showed a positive associated with malaria infection and malaria parasite density, an inverse relationship was demonstrated between other helminth infections and malaria parasite intensity. Children with concurrent *P. falciparum* and *S. mansoni* or any other helminth infection tended to have significantly lower mean malaria parasite densities compared to children infected with *P. falciparum* only. This observation is in line with observations made by Lyke *at al* (2005) and Briand *et al* (2005) in Mali and Senegal, respectively, in studies on malaria and *S. haematobium* co-infection in children aged 4-8 and 3-15 years, respectively. The study of Lyke *et al* (2005) demonstrated that *S. haematobium* infected children had lower geometric mean malaria parasite density compared to children without *S. haematobium* infection. In the study of Briand *et al* (2005) it was shown that children with light infection of *S. haematobium* had lower *P. falciparum* parasite densities compared to those not infected. In an animal model of concormitant *S. mansoni* and *P. berghei* infection, Waknine-Grinberg *et al* (2010) also observed that mice co-infected with *S. mansoni* and *P. berghei* had lower *S. mansoni* egg loads than mice infected with *S. mansoni* only. Understanding of mechanisms behind the observed interactions is still limited (Briand *et al*, 2005), but one possible explanation could be cross reactivity between anti-*P. falciparum* antibodies and anti-schostosomal antibodies. Protective immune response against *P. falciparum* is dominated by IgG1 and IgG3 antibody

subclasses while protective immunity against *S. mansoni* is dominated by IgG1, IgG4, IgGE and to a less extent by IgG3 (Helmby *et al*, 2007; Khalife, 2000). Cross reactivity has been reported for these *S. mansoni* and *P. falciparum* specific antibodies (Helmby *et al*, 2007; Naus *et al*; 2003; Pierrot *et al*, 2007). Furthermore, Concurrent *P. falciparum* infection has been reported to significantly increase production of *S. haematobium* specific IgG3 and IgGE although no cross reactivity was reported (Mutapi *et al*, 2000; Remoue *et al*, 2003). If cross reactivity occurs, anti-schistosome IgG1 and IgG3 which are also protective in blood stage *P. falciparum* infection could easily boost a reaction directed against *P. falciparum* by *P. falciparum* specific antibodies to cause suppressed malaria parasitaemia in co-infected individuals.

Overall anaemia was prevalent in the study area though at a relatively low level compared to what was reported by the study of Lwambo *et al* (1999) which reported an overall prevalence of anaemia of up to 62.4%. This observation may reflect a changing pattern in prevalence of anaemia and the distribution of helminth infections (prevalence and infection intensity) in the study area. The study of Lwambo *et al* (1999) reported relatively higher prevalence and infection intensities of *S. mansoni, S. haematobium* and hookworm infections. Another possible explanation could be the difference in age distribution of children who participated in the two studies. While the current study enrolled children between 3 to 13 years, the study of Lwambo *et al* (1999) enroled children between 7 to 20 years. Majority of anaemia cases were moderate. Only 16 children (1%) had severe anaemia probably due to the fact that majority of helminth infections reported in this study were light. This observation is in agreement with findings of Lwambo *et al* (1992), Koukounari *et al* (2008) and Ajanga *et al* (2006). In the study of Lwambo *et al* (1992), it was observed that anaemia due to heminth infections is dependent on intensity of infection and that for each endemic community, there is a threshold of infection intensity at which significant reductions in Hb levels and hence anaemia occurs. In the studies of Ajanga *et al* (2006) and Koukounari *et al* 2008, it was demonstrated that only heavy *S. mansoni* infections ($\geq$ 400epg) were associated with the risk of anaemia. Further, the two studies observed that hookworm infection which ocurred more frequently as light infections (mean epg < 2000), was not assoctited with anaemia contrally to previous reports (Olsen *et al*, 1998; Dreyfus *et al*, 2000; Shulman *et al* 1996). Anaemia was found to be more prevalent in boys than girls probably due to the relationship between anaemia and the prevalence and infection intensity of parasitic infections which were also higher in boys than girls. As expected and in accordance with findings of other studies (Stoltzfus *et al*, 1997; Friedman *et al*, 2005; Bhargava *et al*, 2003; Guyatt *et al*, 2001; Lwambo *et al*, 1999; Nacher *et al*, 2001; Stolztfus *et al*, 2000; Olsen *et al*, 1998), lower Hb concentrations and anaemia was associated with single and multiple parasitic infections. Although the aetiology of anaemia is multifactorial, parasitic infections are known to be among major causes (Olsen *et al*, 1998; Brooker *et al*, 1999; Koukounari *et al*, 2008). *P. falciparum* infection causes anaemia through complex mechanisms including destruction of parasitized red blood cells, decreased production of red blood cells (RBCs) and/or dyserythropoiesis (Menendez *et al*, 2000; Pradhan, 2009; Warrel *et al*, 2002). *S. mansoni* and *S. haematobium* infections also cause blood loss as schistosome eggs penetrate the walls of intestinal and urinary tract, respectively (Friedman *et al*, 2005). Hookworm infection causes anaemia through a process of chronic intestinal blood loss (Stoltzfus *et al*, 1997; Brooker *et al*, 1999; Hotez *et al*, 2004). However, in contrast to previous studies (Stephenson *et al*, 1993; Olsen *et al*, 1998; Dreyfuss *et al*, 2000; Ndyomugenyi *et al*, 2008; Guyatt *et al*, 2001; Hotez *et al*, 2004; Lwambo *et al*, 1999; Stoltzfus *et al*, 1996), hookworm was found not to be associated with anaemia probably due to the relatively low infection intensities (geometric mean epg 54, 95% CI 46-63) of hookworm infection detected in the studied population.

Further, multiple logistic regression analysis showed that malaria and *S. haematobium* infections were predictors of anaemia, a finding which indicates that in addition to the known effect of single parasite species on anaemia, multiple parasite infections can interact to enhance the risk of anaemia. Previous studies which observed a combined effect of multiple parasite infections on anaemia includes the study of Stephenson *et al* (1985) in Kisumu district, Kenya, Guyatt *et al* (2001) in Korogwe district, Tanzania and Olsen *et al* (1998) in Kwale district, Kenya. Stephenson *et al* (1985) used a stepwise multivariate regression analysis to examine the relationship between Hb levels and *S. haematobium*, hookworm and malaria infections before and 6 months after treatment with metrifonate and found that decreases in *S. haematobium*, hookworm and malaria parasite counts were important determinants of increase in Hb levels. Likewise, Guyatt *et al* (2001) used logistic regression analysis to study factors affecting the risk of school children being anaemic and found that infection intensity of both hookworm and *S. haematobium* were important predictor variables for anaemia. Olsen *et al* (1998) found that malaria infection was a predictor of low Hb in adult females but not in adults males and children and attributed this to the differences in number of malaria attacks per year between children and adults in relation to the time for recovery of Hb levels between attacks. No explanation was given for lack of association between malaria infection and Hb levels in males. Another study in the Phillipines (Ezeamama *et al* 2005) demonstrated that even at low infection intensity, polyparasite infections involving *S. japonicum, T. trichiura, A. lumbricoides* and *N. americanus* were associated with 5 fold more risk of anaemia in infected children (7-18 years) compared to uninfected children of the same age group. Mechanisms through which malaria, schistosoiasis and STH independently cause anaemia have been described. These results show that in multiple species infections, there is a possibility of different mechanisms in the causal pathway of anaemia to interact and cause enhanced risk of anaemia.

Interestingly, the highest prevalence of anaemia (60%) was observed in children concurrently infected with *P. falciparum*, *S. mansoni* and *S. haematobium,* and in children concurrently infected with *P. falciparum* and *S. haematobium* (52.3%). Anaemia was also more prevalent in children concurrently infected with three or four parasites compared to those with only one or no parasite infection. These observations demonstrate a possible synergistic interaction of *P. falciparum, S. mansoni* and *S. haematobium* and multiple parasite infections as the aetiology of anaemia. The observation also provides further evidence in favour of the hypothesis that co-infected individuals have an increased risk of morbidity compared to uninfected individuals and those infected with only one parasite species. Similar findings have been reported by other studies (Stoltzfus *et al*, 1997; Nacher *et al*, 2002; Basavaraju and Schwantz, 2006; Tshikuka *et al*, 1996; Torre *et al*, 2002).

Overall, results of this study have shown that malaria, schistosomiasis and STH infections are prevalent in school and pre-school children in Magu district and that polyparasitism is also very common. The most important parasite combinations involved *S. mansoni, P. falciparum* and *S. haematobium*. STH are also common though with relatively low prevalence. These results also suggest that while hookworm infection increases the likelihood of *P. falciparum* infection, *S. mansoni* and *S. haematobium* infection tends to protect against severe consequences of *P. falciparum* infection through a counter effect on malaria parasite multiplication. Further, the study provided clear evidence that concurrent *P. falciparum*, *S. mansoni* and *S. haematobium* infection enhance the risk of lower Hb levels and anaemia. These results suggest that helminth infections could influence malaria transmission and malaria related morbidity. Further, the results show the importance of assessing for the presence of multiple parasite infections and related morbidity in endemic communities.

3.5. References

Ajanga, A., Lwambo, N. J. S., Blair, L., Nyandindi, U., Fenwick, A., Brooker, A. (2006). *Schistosoma mansoni* in pregnancy and association with naemia in Nortwest Tanzania. *Trans. Roy. Soc. Trop. Med. Hyg,* **100:** 59-63.

Archie C. A. C., Lwambo, N J S., Blair, L., Nyandindi, U., Godfrey Kaatano, G., Safari Kinung'hi., Webster, J., Fenwick, A., Brooker, S. (2006). Bayesian spatial analysis and disease mapping: Toola to enhance planning and implementation of schistosomiasis control programme in Tanzania. *Trop Med Int Health*, **4:** 490-503.

Basavaraju, S. V and Schantz. P. (2006). Soil-transmitted helminthes and plasmodium falciparum malaria: Epidemiology, clinical manifestation and the role of nitric oxide in malaria and geohelmiths co-infections. Do worms have a protetive role in P. falciparum infection? *Mount Sinai J Med,* **73** (8): 1098-1105.

Bhargava, A., Jukes, M., Lambo, J., Kihamia, C. M., Lorri, W., Nokes, C., Drake, L., Bundy, D. (2003). Anthelmintic treatment improves the hemoglobin and serum ferritin concentrations of Tanzanian schoolchildren. *Food Nutr Bull*, **24** (4): 332 - 342.

Booth, M., Vennervald, B. J., Kenty, L., Butterworth, A. E., Kariuki, H. C., Kadzo, H., Amaganga, C., Kimani, G., Mwatha, J. K., Otedo, A., Ouma, J. H., Muchiri, E., Dunne, D. W. (2004). Micro-geographical variation in exposure to *S. mansoni* and malaria, and exacerbation of splenomegally in Kenyan School age children. *BMC Infect Dis*, **4:** 13.

Briand, V., Laurence Watier., Jean-Yves Le Hesran., Andre Garcia and Michel Cot (2005). Co-infection with *Plasmodium falciparum* and *Schostosoma haematoboum*: Protective effect of Schostosomiasis on malaria in Senegalese children? *Am. J. Trop. Med. Hyg*, **72:** 702-707.

Brooker, S., Peshu, N., Warn, P. A., Mosobo, M., Guyatt, H. L., Marsh, K., Snow, R. W. (1999). The epidemiology of hookworm infection and its contribution to anaemia among pre-school children on the Kenyan coast. *Trans. R. Soc. Trop. Med. Hyg.* **93** (3): 240-246.

Brooker, S., Miguel, E. A., Moulin, S., Luoba, A. I., Bundy, D. A., Kremer, M. (2000). Epidemiology of single and multiple species of helminth infections among school children in Busia District, Kenya. *East Afr Med J*, **77** (3): 157-61.

Brooker, S., Akhwale, W., Pullan, R., Estambale, B., Clarke, S. E., Snow, R. W., Hotez, P. J. (2007). Epidemiology of plasmodium-helminth co-infection in Africa: Populations at risk, potential impact on anemia, and prospects for combining control. *Am. J. Trop. Med. Hyg,* **77:** 88-98.

Brooker, S., Kabatereine, n. B., Smith, J. L., Mupfasoni, D., Mwanje, M. T., Ndayishimiwi, O., Lwambo, N. J. S., Mbotha, D., Karanja, Peris., Mwandawiro, C., Muchiri, E., Clements, A. C. A., Bundy, D. A., Snow, R. W. (2009). An updated atlas of human helminth infections: the example of East Africa**.** *Intl J Health Geogr,* **8**: 42.

De Silva, N. R., Brooker, S., Hotez, P. J., Montresor, A., Engels, D., Savioli, L. (2003). Soil transmitted helminth infections: Updating the global picture. *Trends Parasitol*, **19**: 547-551.

Dreyfuss, M. L., Stoltzfus, R. J., Shrestha, J. B., Pradhan, E. K., Leclerg, S. C., Khatri, S. K., Shresha, S. R., Katz. J., Albonico, M., West, K. P. (2000). Hookworms, malaria and vitamin A deficiency contributes to anaemia and iron deficiency among pregnant women of Nepal. *J Nutr*, **130:** 2527-2536.

Egwunyenga, O. A., Ajayi, J. A., Nmorsi, O. P. G., Duhlinska-Popova, D. D. (2001). Plasmodium/intestial helminth co-infections among pregnant Nigerian women. *Mem. Inst. Oswaldo Cruz*, **96** (8): 1055-1059.

Ezeamama, A. E., Friedman, J. F., Acosta, L. P., Bellinger, D. C., Langdon, G. C., Manalo, D. L., Olveda, R. M., Kurtis, J. D., McGarvey, S. T. (2005). Helminth infection and cognitive impairment among Filipino children. *Am J Trop Med Hyg*, **72** (5): 540-548.

Friedman, J. F., Kanzaria, H. K., McGarvey, S. T. (2005). Human schistosomiasis and anemia: the relationship and potential mechanisms. *Trends Parasitol,* **21** (8): 386-392.

Guyatt, H. L., Brooker, S., Kihamia, C. M., Hall, A., Bundy, D. A. (2001). Evaluation of efficacy of school-based anthelmintic treatments against anaemia in children in the United Republic of Tanzania. *Bull World Health Organ,* **79** (8): 695-703.

Helmby, H., Kullberg, M., Troye-Blomberg, M. (1998). Altered immune responses in mice with concomitant *Schistosoma mansoni* and *Plasmodium chabaudi* infections. *Infect Immun*, **66** (11): 5167-5174.

Helmby, H. (2007). Schistosomiasis and Malaria: Another piece of cross-reactivity puzzle. *Trends Parasitol.* **23** (3): 88-90.

Hillier, S. D., Booth, M., Muhangi, L., Nkurunziza, P., Khihembo, M., Kahande, M., Sewankambo, M., Kizindo, R., Kiza, M., Kiwanga, M., Elliot, A. M. (2008). Plasmodium falciparum and helminth coi-infections in semiurban population of pregnant womenin Ugnada. *J. Infect. Dis,* **198**: 920-927.

Hotez, P. J., Brooker, S., Bethony, J. M. (2004). Current concepts: Hookworm infection. *N Engl J Med,* **351** (8): 799-808.

Howard, S. C., Donnelley, C. A., Chan, M.-S. (2001). Methods for the estimation of associations between multiple species parasite infections. *Parasitol*, **122:** 233-251.

Howard, S. C., Donnelley, C. A., Kabatereine, N. B., Ratard, R. C., Brooker, S. (2002). Spatial and intensity dependent variation in associations between multiple species helminth infections. *Acta Trop,* **83:** 41-149.

Kabatereine, N. B., Kemijumbi J., Ouma J. H., Kariuki H. C., Richter J., Kadzo H., Madsen H., Butterworth A. E, Ørnbjerg N., Vennervald B J. (2004). Epidemiology and morbidity of *Schistosoma mansoni* infection in a fishing community along Lake Albert in Uganda. *Trans Roy Soc Trop Med Hyg,* **98** (12): 711-718.

Keizer, J., N'Goran, E., Traore, M., Lohourignon, K. L., Singer, B. H., Lengeler, C., Tanner, M., Utzinger, (2002). Polyparasitism with *Schistosoma mansoni*, geohelminths and intestinal protozoa in rural Cote D'Ivoire. *J. Parasitol,* **88** (3): 461-466.

Khalife, J., Catherine C., Pierrot, C., Capron, M. (2000). Mechanisms of resistance to *S. mansoni* infecction: The rat model. *Parasitolo Intl*, **49** (2000), 339-345.

Koukounari, A., Estambale, B. B. A., Njagi, J. J., Cundill, B., Ajanga, A., Crudder, C., Otido, J., Jukes, M. C. H., Clarke, S. E., Brooker, S. (2008). Relationship between anaemia and parasitic infections in Kenyan school children. *Intel Jl Parasitol,* **38:** 1663-1671.

Latham, M. C., Stephenson, L. S., Kurz, K. M., Kinoti, S. N. (1990). Metrifonate or praziquantel treatment improves physical fitness and appetite of Kenyan schoolboys with schoistosoma haematobium and hookworm infections. *Am. J. Trp. Med. Hyg,* **43:** 170-179.

Lwambo, N J. S., Bundy, D. A. P., Medley, G. F. H. (1992). A new approach to morbidity risk assessment in hookworm endemic countries. *Epidemiol. Infect,* **108:** 469-481.

Lwambo, N. J. S., Siza, J. E., Brooker, S., Bundy, D. A. P., Guyatt, H. (1999). Patterns of concurrent hookworm infections and schistosomiasis in school children in Tanzania. *Trans. Roy. Soc. Trop. Med. Hyg,* **93:** 497-502.

Lyke, K. E., Dicko, A., Bado, A., Sangare, L., Kone, A., Coulibaly, D., Guindo., A., Traore, K., Daou, M., Diara, Issa., Sztein, M., Plowe, C. V., Doumbo, O. K. (2005). Association of *Schistosoma haematobium* infection with protection against acute *Plasmodium falciparum* malaria in Malian children. *Am. J. Trop Med. Hyg,* **73** (6): 1124-1130.

Magnussen, P., Ndawi, B., Sheshe, A. K., Byskov, J., Mbwana, K., Christensen, N. O. (2001). The Impact of a school health programme on the prevalence and morbidity of urinary schistosomiasis in Mwera Division, Pangani District, Tanzania. *Trans Roy Soc Trop Med Hyg,* **95** (1): 58-64.

Magnussen, P., Ndawi, B., Sheshe, A. K., Byskov, J., Mbwana, K. (2002). Prevalence and morbidity of urinary schistosomiasis among school children in the Mwera Division of Pangani district, Tanazania. *Ann Trop Med Parasitol,* **96** (8): 843-848.

Maizels R. M., Balic A., Gomez-Escobar N., Nair M., Taylor M. D., Allen J. E. (2004). Helminth parasites–masters of regulation. *Immunol Rev,* **201***:* 89–116.

Mboera, L. E. G and Kitua, A. Y. (2001): Malaria epidemics in Tanzania. *African J Health Sci,* **8**: 14-18.

Mboera, L. E. G. (2004): Environmental and socio-economic determinants of malaria epidemics in highlands of Tanzania. *Tanzania Health Res Bull,* **6**: 11-17.

Menendez, C., Fleming, A. F., Alonso, P. L. (2000). Malaria related anaemia. *Parasitol. Today*, **16:** 469-476.

Mutapi, F., Ndlovu, P. D., Hagan, P., Woolhouse, M. E. J. (2000). Anti-schistosome antibody responses in children co-infected with malaria. *Parasite immunology*, **22:** 207-209.

Mwangi, T. W., Bethony, J. M., Brooker, S (2006). Malaria and helminth interactions in humans: an epidemiological viewpoint. *Ann Trop Med Parasitol*, **100 (**7): 551-570.

Nacher, M., Singhasivanon, P. P., Silachamroon, U., Treeprasertsuk, S., Krudsood, S., Gay, F., Mazier., Looareensuwan, S. (2001). Association of helminth infections with increased gametocyte carriage during mild falciparum malaria in Thailand. *Am. J. Trop. Med. Hyg,* **65** (5): 644-647.

Nacher, M., Singhasivanon, P., Yimsamran, S., Manibunyong, W., Thanyavanich, N., Wuthisen, P., Looareesuwan, S (2002). Intestinal helminth infections are associated with increased incidence of *Plasmodium falciparum* malaria in Thailand. *J Parasitol*, **88** (1): 55-58.

Naus, C. W. A., Jones, F. M., Satti, M. Z., Joseph, S., Riley, E. M., Kimani, G., Mwatha, J. K., Kariuki, C. H., Ouma, J. H., Kabatereine, N. B., Vennervald, B. J (2003). Serological responses among individuals in areas where both schistosomiais and malaria are endemic: Cross reactivity between schistosoma mansoni and *Plasmodium falciparum*. *J. Infect. Dis,* **187:** 1272-1282.

Ndyomugyenyi R., Kabatereine N., Olsen A., Magnussen, P. (2008). Malaria and hookworm infections in relation to haemoglobin and serum ferritin levels in pregnancy in Masindi district, western Uganda. *Trans R Soc Trop Med Hyg,* **102:** 130-136.

Nkuo-Akenji, T. K., Chi, P. C., Cho, J. F., Ndamukong, K. J., Sumbele, I. (2006). Malaria and helminth co-infections in children living in a malaria endemic setting of mount Cameroon and predictors of anaemia. *J. Parasitol,* **92** (6): 1191-1195.

Olsen, A., Magnussen, P., Ouma, J. H., Andreassen, J., Friis, H. (1998). The contribution of hookworm and other parasitic infections to haemoglobin and iron status among children and adults in western Kenya. *Trans Roy Soc Trop Med Hyg,* **92** (6): 643-649.

Partney, T. N., Andrews, R. H. (1998). Multiparasite communities in animals and humans. Frequency, structure and pathogenic significance. *Int J. Parasitol,* **28:** 377-393.

Pierrot, C., Wilson, S., Lallet, H., Laffite, S., Jones, F. M., Daher, W., Capron, M., Dunne, D. W., Khalife, J. (2006). Identification of a novel Antigen of S*chistosoma mansoni* shared with P*lasmodium falciparum* and evaluation of different cross-reactive antibody subclass induced by human schistosomiasis and malaria. *Infect Immun,* **72:** 3347-3354.

Pradhan, P. (2009). Malaria anaemia and nitric oxide induced megaloblastic anaemia: A review on the causes of malarial anaemia. *J. Vector Borne Dis,* **46:** 100-108.

Remoue, F., Dialo, T. O., Angeli, V., Herve, M., De Clercq, D., Schacht, A. M., Charrier, N., Capron, M., Vercruysse, J., Ly, A., Capron, A., Riveau, G. (2003). Malaria co-infection in children influences antibody response to schistosome antigen and inflammatory markers associated with morbidity. *Trans. Roy. Soc. Trop Med. Hyg,* **97:** 361-364.

Shapiro, A. E., Tukahebwa, E. M., Kasten, J., Clerk, S., Magnussen, P., Olsen, A., Kabatereine, N. B., Ndyomugenyi, R., Brooker, S. (2005). Epidemiology of helminth infections and their relationship to clinical malaria in Southwest Uganda. *Trans. Roy. Soc. Trop. Med. Hyg,* **99:** 18-24.

Shulman, C. E., Graham, W. J., Jilo, H., Lowe, B. S., New, L., Obiero, J., Snow, R. W., Marsh, K. (1996). Malaria is an important cause of anaemia in primigravidae: Evidence from a district hospital in coastal Kenya. *Trans. Roy. Soc. Trop. Med. Hyg,* **90:** 535-539.

Sokhna, C., Le Hesran, J. Y., Mbaye, P. A., Akiana, J., Camara, P., Diop, M., Ly, A., Druilhe, P. (2004). Increased malaria attacks among children presenting concomitant infections by *S. mamsoni* in Senegal. *Mal Jl*, **3:** 43.

Snow, R W., Guera, C. A., Noor, A. M., Myint, H. Y., Hay, H. I. (2005). The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature*, **434:** 214-217.

Spiegel, A., Tall, A., Raphenon, G., Trape, J. F., Druilhe, P. (2003). Increased frequency of malaria attacks in subjects co-infected by intestinal worms and *Plasmodium falciparum* malaria. *Trans. Roy. Soc. Trop. Med. Hyg,* **97:** 198-199.

Stephenson, L. S., Latham, M. C., Kurtz, K. M., Kinoti, S. N., Oduori, M. L., Crompton, D. W. T. (1985). Relationship of *Schistosoma haematobium*, hookworm and malarial infections and metrifonate treatment to haemoglobin levels in Kenyan school children. *Am. J. Trop. Med. Hyg,* **34:** 519-527.

Stoltzfus, R. J., Albanico, M., Chwaya, H. M., Savioli, L., Tielsch, J., Schulze, K., Yip, R. (1996). Haemoquant determination of hookworm related blood loss and its role in iron deficiency in African children. *Am. J. Trop. Med. Hyg,* **55:** 399-404.

Stoltzfus R. J., Chwaya H. M., Tielsch J. M., Schulze K. J., Albonico M., Savioli L. (1997). Epidemiology of iron deficiency anemia in Zanzibari schoolchildren: the importance of hookworms. *Am J Clin Nutr,* 65 (1): **153-159**.

Stoltzfus, R. J., Chwaya, H. M., Montresor, A., Albonico, M., Savioli, L., Tielsch, J. M. (2000). Malaria, hookworms and recent fever are related to anemia and iron status indicators in 0 to 5 year old Zanzibari children and these relationships change with age. *J. Nutr,* **130** (7): 1724-1733.

Tchuem-Tchuente L. A., Behnke, J. M., Gilbert, F. S., Southgate, V. R., Vercruisse, J. (2003). Polyparasitism with *schistosoma haematobium* and soil-transmitted helminth infections among school children in among school children in Loam, Cameroon. *Trop Med. Int. Health,* **8:** 975-986.

Torre, D., Speranza, F., Martegani, R. (2002). Role of pro-inflammatory and anti-inflammatory cytokines in the immune response to *Plasmodium falciparum* malaria. *Lancet Infect Dis,* **2** (12): 719-720.

Tshikuka, J. G., Scott, M. E., Gray-Donald, K., Kalumba, O. N. (1996). Multiple infection with plasmodium and helminths in communities of low and relatively high socio-economic status. *Ann Trop Med Parasitol*, **90:** 277-293.

Waknine-Grinberg, J. H., Gold, D., Ohayon, A., Flescher, E., Heyfts, A., Doenhoff, M. J., Schramm, G., Haas, H., Golenser, J. (2010). Schistosoma mansoni infection reduces the incidence of cerebral malaria. *Mal J*, **9**: 5

Warrel, D. A., Turner, G. D. H., Francis, N. (2002). Pathology and pathophysiology of human malaria. In: Warrel, D. A and Gilles, H. M (2002). Essential malariology. 4th Edition, Arnold Publishers, London: UK. Pp 236- 251.

Webbe, G. (1962). The transmission of *Schistosoma haematobium* in an area of Lake Province, Tanganyika. *Bull World Health Organ,* **27**: 59-85.

WHO (1991). Basic laboratory methods in medical parasitology. WHO, 1991. Geneva, Italy.

WHO (1994). Bench aids for the diagnosis of intestinal parasites. WHO, Geneva, Italy.

Yoshida, A., Maruyama, H., Kumagai, T., Amano, T., Kobayashi, F., Zhang, M., Himeno, K., Ohta, N. (2000). *Schistosoma mansoni* infection cancels the susceptibility to *Plasmodium chabaudi* through induction of type 1 immune responses in A/J mice. *Intl immunol*, **12**: 1117-1125.

Chapter 4: Clinical and ultrasonographic observations in school and pre-school children in an area endemic for schistosomiasis and malaria in Magu district, Northwestern Tanzania

Abstract

Infection with *schistosoma* species is very common among school and pre-school children in schistosomiasis endemic countries and is associated with pathology which may occur at a very early age. Pathology results from schistosome eggs trapped in host tissues which become surrounded by inflammatory cells resulting in granulomatous reactions followed by fibrosis. The current study assessed *S. mansoni* and *S. haematobium* related morbidity in 1546 school and pre-school children in Magu district, Northwestern Tanzania. Clinical and ultrasound examinations were performed for all children according to standardized protocols. Clinically, enlargement of the liver and/or spleen was detected in 98 children (6.3%). Periportal fibrosis (PPF) was detected in 35 children (2.3%) of whom 6 had advanced PPF. Enlargement of the liver, spleen and portal vein diameter (PVD) was detected in 79.1%, 39.9% and 15.2% of all children examined with ultrasound, respectively. There was a significant association between prevalence of PPF and age group ($\chi^2 = 7.81$, $p = 0.020$). *S. mansoni* infection intensity was significantly associated with prevalence ($\chi^2 = 8.89$, $p < 0.001$) and severity ($F = 7.16$, $p < 0.001$) of PPF. The prevalence of hepatomegaly correlated with intensity of *S. mansoni* infection, being higher in children with moderate to heavy *S. mansoni* infection compared to children with light or no *S. mansoni* infection ($\chi^2 = 6.84$, $p = 0.033$). The prevalence of splenomegaly was significantly higher in boys (42.7%) than in girls (37.2%) ($\chi^2 = 4.80$, $p = 0.028$), and increased with age ($\chi^2 = 50.05$, $p < 0.001$). Splenomagely was significantly associated with *P. falciparum* infection intensity ($\chi^2 = 133.47$, $p < 0.001$). Further, children infected with *P. falciparum* and those concurrently infected with *P. falciparum* and *S. mansoni* had significantly larger spleens compared to uninfected children ($p < 0.001$). The size of PVD correlated with intensity of *S. mansoni* infection whereby children with moderate to heavy *S. mansoni* infection had significantly larger PVD compared to children with light or no *S. mansoni* infection ($F = 17.63$, $p < 0.001$). Overall, children infected with two or more parasites had significantly larger organs (liver, spleen and PVD) compared to children without any parasite infection or those with single parasite infections ($p < 0.05$). Sixty seven children (4.3%) had mild to moderate *S. haematobium* related lesions the majority of which occurred in the lower urinary tract. The prevalence of urinary tract lesions was significantly higher in boys (7.0%) than girls (1.8%) ($\chi^2 = 25.24$, $p < 0.001$) and increased with age ($\chi^2 = 19.46$, $p < 0.001$). The prevalence of urinary tract pathology differed significantly among schools ($\chi^2 = 19.62$, $p < 0.01$) reflecting variation in transmission and intensity of *S. haematobium* infection. The prevalence and severity of urinary tract pathology correlated with intensity of *S. haematobium* infection whereby children with heavy *S. haematobium* infection had significantly more prevalent and severe urinary tract pathology compared to children with light or no *S. haematobium* infection ($\chi^2 = 157.72$, $p < 0.001$). In the multivariate analysis, *S. mansoni* infection intensity and area of residence were significant predictors of both PPF and enlarged left liver lobe. Age group, malaria parasite density and hookworm infection were significant predictors of enlarged spleens. Malaria parasite density and hookworm infection were significant predictors for enlargement of both the liver and spleen. Age, sex, hookworm infection and infection intensity of *S. haematobium* were significant predictors of urinary tract pathology. In conclusion, this study demonstrated that schistosome related morbidity of the liver, spleen and urinary

tract is common in school and pre-school children in the study area and is positively correlated with both the prevalence and infection intensity of *S. mansoni* and *S. haematobium,* respectively. These findings demonstrates the importance of morbidity assessment using ultrasound as a means of disease surveillance and evaluation of the impact of schistosomiasis control programmes in school and pre-school children.

4.1. Introduction

Pathological effects due to infection with schistosoma species are very common in schistosomiasis endemic countries and may occur at an early age (Fulford *et al*, 1991; Wilson *et al*, 2007: Butterworth *et al*, 1994; Stothard and Gabrielli, 2007). Pathology result from schistosome eggs trapped in host tissues which become surrounded by inflammatory cells resulting in granulomatous reactions which may be followed by fibrosis (Davis, 2009; Abdel-Wahab and Mahmoud, 1987). Soluble egg antigens (SEAs) originating from the secretory glands of miracidia enclosed within schistosome eggs diffuse out through submicroscopic pores in the eggshell and induce a host hypersensitivity response. The immunopathology of schistosomiasis is therefore due to granuloma formation around tissue-deposited eggs and is a manifestation of delayed hypersensitivity through a T cell-mediated immune response (Davis, 2009). A typical schistosome granuloma is composed of the schistosome egg surrounded by cellular aggregates of eosinophils, mononuclear phagocytes, lymphocytes, neutrophils, plasma cells and fibroblasts. Activated macrophages cluster close to the eggshell, while lymphocytes and plasma cells are peripherally placed (Davis, 2009). In *S. mansoni* infection, eggs are deposited in the submucosa and subserosa and sometimes in the muscle layer of the colon from where they are carried by the portal circulation to the liver where they induce vascular inflammatory and granulomatous changes (Davis, 2009). Pseudo tubercle formation and portal fibrosis may occur which leads to increased portal pressure (portal hypertension) which manifests itself as gastroesophageal varices, portal systemic collaterals and dilation of abdominal wall veins. This in turn may lead to life threatening haematemesis and/or melaena due to rupture of gastroesophagial varices. As the disease advances ascites and oedema develops (Lambertucci *et al*, 1993; Abdel-Wahab and Mahmoud, 1987). *S. mansoni* infection is characterized by large intestine involvement with abdominal tenderness, abdominal pain, diarrhoea or dysentery. There may be passage of blood and mucus in stool leading to anaemia (Lambertucci, 1993; Abdel-Wahab and Mahmoud, 1987). Enlargement of the liver and spleen (hepatosplenomegaly) is another frequent clinical manifestation of *S. mansoni* infection and is a common accompaniment of hepatosplenic disease. Hepatosplenomegaly is caused by portal venous congestion and hyperplasia of reticuloendothelial cells. It is usually accompanied by marked firmness or even hardness of the affected organs. The enlarged liver, particularly the left lobe becomes smooth, firm and non-tender. The spleen may become greatly enlarged, sometimes extending downwards past the umbilicus into the left iliac fossa (Vennervald *et al*, 2004; Davis, 2009).

For *S. haematobium* infection, target organs include the urinary bladder, ureters, kidneys and genital organs (Davis, 2009). *S. haematobium* eggs are trapped in the bladder wall and ureters where they induce formation of granulomas. The eggs become calcified and surrounded by fibrous tissues and infiltration with inflammatory cells occurs (Davis, 2009). Advanced heavy infections causes extensive hyperplasia of inner epithelial cells of the bladder and urethra resulting into lower urinary tract lesions such as bladder wall irregularities, masses, pseudopolyps and calcifications. All these changes lead to

distortion of bladder shape and structure and reduction of bladder capacity. Eggs deposited in ureter walls produce fibrosis which causes ureteric stenosis and obstruction resulting in dilatation of ureters (hydroureter) and hydronephrosis (Davis, 2009; Lambertucci *et al*, 1993). *S. haematobium* eggs may also be deposited in the cervix, vulva, ovaries and fallopian tubes and male genital organs causing genital schistosomiasis. Macrohaematuria is a pathognomonic clinical manifestation of advanced *S. haematobium* infection (Davis, 2009; Ukoli, 1984).

In areas where both *S. mansoni* and *P. falciparum* are co-endemic, coinfections with these parasite species are common particularly in school age children. Both *S. mansoni* and *P. falciparum* infections cause enlargement of the liver and/or spleen. Studies have shown that the severity of hepatosplenic schistosomiasis correlates well with *S. mansoni* infection intensity indicating a strong association between organ enlargement and schistosome infection (Sowunmi, 1996; Vennervald *et al*, 2004). Further, studies have also shown that there is evidence of involvement of both *S. mansoni* and malaria infection on the degree of hepatosplenic disease (Booth *et al*, 2004a; Mwatha *et al*, 2003). Results from a study conducted in Kenya combining clinical and ultrasound examination with spatial analysis of microgeographical variation in exposure to *S. mansoni* and malaria showed that hepatosplenic disease was more severe in children living in areas of higher exposure to both *S. mansoni* and malaria compared to children exposed to either infection alone (Booth *et al*, 2004b). Concurrent exposure to these two parasites may therefore result in a higher prevalence and severity of hepatosplenomegaly (Whittle *et al*, 1969; Fulford *et al*, 1991; Booth *et al*, 2004b; Wilson *et al*, 2007).

While malaria-helminth co-infections are common in SSA, little is known about their impact on severity of disease and pathology. There is therefore a need for a more thorough understanding of morbidity patterns in persons with schistosomiasis and malaria co-infections and the impact of treatment. The objective of this part of the study was to assess patterns of schistosomiasis related morbidity in school and pre-school children in Magu district, an area where both schistosomiasis and malaria are endemic.

4.2. Methodology

4.2.1. Study area and population

The study was conducted in Magu district, Tanzania. Details of the study area and population have been given in chapter 2. Briefly, 1546 school and pre-school children from 6 schools/villages aged 3 – 13 years were included in the study. All schools selected are located in close proximity to the shoreline of Lake Victoria along the main tarmac road running from Mwanza city to Nairobi. Lake Victoria is the main source of water for domestic use in this area. Inhabitants of this area are ethnic Sukuma who practice subsistence farming, livestock production, fishing and small scale businesses.

4.2.2. Clinical examination

Clinical examinations were performed by an experienced physician. Measurements recorded included liver tenderness, presence of any palpable liver irregularities, the extension of the left liver lobe beneath the sternum measured in centimetres in the mid-sternal line (MSL), the extension of the right liver lobe

beneath the rib cage, measured in centimetres in the right mid-clavicular line (MCL), the extension of the spleen below the rib cage, measured in centimetres in the left mid-clavicular line (MCL) and left mid-axillary line (MAL) using a measuring tape. An organ was considered enlarged if it was palpable $\geq$ 2 cm below the coastal line. Other measurements included the consistency of the liver and spleen graded as not palpable, soft, firm and hard. Clinical signs of portal hypertension, including ascites and umbilical collaterals, and other findings such as abdominal swellings or scars were also examined. The body temperature of each child was recorded using a digital thermometer.

4.2.3. Ultrasound examination

All children were examined using a portable ultrasound machine (Aloka Sonocamera SSD-500 with 3.5 MHz curvilinear probe) which was supplied with power from a portable generator. The examinations were performed according to the Niamey protocol (Richter *et al*, 2000). Measurements involved the size of the left liver lobe (Liver PSLin cm), the portal vein diameter (PVD in mm) and spleen length (in cm). The liver image pattern was also assessed. For urinary schistosomiasis, ultrasonography examination of the urinary tract (bladder, ureters and kidneys) were performed using the same equipment and protocol (as described above) after the children had been given drinking water to fill the bladder. All pathological changes observed were recorded and graded as described in detail in chapter 2.

4.2.4. Data analysis

Periportal fibrosis (PPF) due to *S. mansoni* infection based on observed liver image pattern (IP) was classified as normal (IP = A-B), mild to moderate (IP = C-D) and severe (IP = E-F). Pathologocal changes due to *S. haematobium* infection were classified as "normal" if no lesion was detected; "mild" if focal bladder wall irregularity and/or bladder wall thickening was observed; "moderate" if diffuse bladder wall irregularity and/or bladder wall thickening was observed or one outgrowth of bladder wall (mass/pseudopolyps) or dilation of ureters was observed; "severe/advanced" if calcification of bladder wall or more than one outgrowth of bladder wall (mass/pseudopolyps) or marked dilation of ureters and/or hydronephrosis was observed. Data from children with incomplete parasitological or ultrasonographic information were excluded from analysis. Analysis of height standardized ultrasound data was carried out as described by Richter *et al*, 2000. Study participants were classified into four height groups (80-100cm, 101-120cm, 121-140cm and 141-160cm). The values for the left liver lobe (liver PSL), spleen length and portal vein diameter (PVD) were classified as "normal" if they were below or equal to the mean $\pm$ 2SD; "moderately abnormal" if they were more than 2 SD but below or equal to the mean $\pm$ 4 SD and "abnormal" if they were above the mean $\pm$ 4 SD of the values for the corresponding height groups from a Senegalese non-infected population as suggested by Richter *et al*, 2000. Information collected was double entered into Dbase V software (Borland International, Scotts Valley, California, USA). Descriptive and advanced analysis was performed using STATA version 10 (STATA Cooperation, Texas, USA). Infection intensities were calculated as geometric means of eggs per gram of faeces for *S. mansoni* and hookworm infections, eggs per 10ml of urine for *S. haematobium* and parasites per microlitre of blood for *P. falciparum* among positives only. The student's t-test and one way analysis of variance (ANOVA) was used to compare mean size of left liver

lobe (in PSL), spleen size (spleen length in cm), PVD and geometric mean parasite counts where two or more than two groups were compared, respectively. The Chi-square test was used to compare proportions and to test for association between outcome and exposure variables. Multivariate regression analysis was used to determine significant predictors of PPF, hepatomegaly, splenomegaly, hepatosplenomegaly and urinary tract pathology. Graphs were drawn using STATA 10 or MS-Excel as appropriate. Tests were considered statistically significant at $p < 0.05$.

4.3. Results

4.3.1. Prevalence of organ enlargement as detected at clinical examination

Out of 1546 children examined, 759 (49.1%) were boys. Mean age was 7 years. Overall organ enlargement (liver and/or spleen) was detected in 98 (6.3%) children. Out of the 98 children, enlargement of the liver only (hepatomegaly) was detected in 4 children while enlargement of the spleen only (splenomegaly) was detected in 90 children. Only 4 children had hepatosplenomegaly. Most of enlarged organs were of soft consistence, hard or firm liver or spleen was recorded in 18 children only (1.2%). Six out of the eight children who had enlarged livers were excreating *S. mansoni* eggs while all four children who had enlargement of both the liver and spleen were excreating *S. mansoni* eggs. The four children were also anaemic and two of them were infected with *P. falciparum*.

4.3.2. Prevalence of pathology as detected by ultrasonography

Out of the 1546 children examined, 1,511 children (97.7%) had normal liver texture patterns (IP A – B) while 35 children (2.3%) had abnormal liver texture patterns. One child with liver image pattern E had collaterals. The prevalence of *S. mansoni* related pathology is shown in Table 4.1.

Table 4.1 Prevalence of *S. mansoni* related pathology (by sex) as detected by ultrasound (n = 1546).

Type of pathology	**Number with pathology (%)**		
	Normal	**Mild/moderate**	**severe/advanced**
Periportal fibrosis			
Total	1511 (97.7)	33 (2.2)	2 (0.1)
Boys	740 (97.5)	18 (2.3)	1 (0.1)
Girls	771 (98.0)	15 (2.0)	1 (0.1)
P-Value	0.534	0.527	NA
Enlargement of left liver lobe			
Total	325 (21.0)	681 (44.1)	540 (35.0)
Boys	157 (20.7)	333 (43.9)	269 (35.4)
Girls	168 (21.4)	348 (44.2)	271 (34.4)
P-Value	0.750	0.891	0.678
Enlargement of the spleen			
Total	929 (60.1)	519 (33.6)	98 (6.3)
Boys	435 (57.3)	277 (36.5)	47 (6.2)
Girls	494 (62.8)	242 (30.8)	51 (6.5)
P-Value	0.028	0.017	0.816
Enlargement of portal vein diameter (PVD)			
Total	1,311 (84.8)	227 (14.7)	8 (0.5)
Boys	631 (83.1)	121 (15.9)	7 (1.0)
Girls	860 (86.4)	106 (13.5)	1 (0.1)
P-Value	0.074	0.170	NA

P-Values refer to differences between boys and girls, NA means not applicable

Figure 4.1 Subcostal liver scan showing periportal fibrosis (arrows) (liver IP = E) due to *S. mansoni* infection.

There was no significant difference in the prevalence of periportal fibrosis between boys and girls (χ^2 = 0.39, p = 0.534). The prevalence of PPF increased with age whereby it was significantly higher (3.4%) in children aged 9 – 13 years compared to those aged 6 – 8 years (2.8%) and those aged 3 – 5 years (0.3%) (χ^2 = 7.81, p = 0.020). The youngest child with PPF (liver IP = C) was a boy aged 5 years from Mwamayombo primary school, one of the schools with relatively higher prevalence and infection intensity of *S. mansoni*. There was no significant difference in prevalence of enlarged left liver lobe among age groups (χ^2 = 0.10, p = 0.750). The prevalence of enlarged spleens increased significantly with age, being higher (56.7%) in children aged 9 – 13 years compared to 40.1% in children aged 6 – 8 years and 27.3% in children aged 3 – 5 years (χ^2 = 50.10, p < 0.001). Five hundred children (32.3%) had enlargement of both the liver and the spleen (hepatosplenomegaly). The prevalence of hepatosplenomegaly was 34.8% in boys and 30.0% in girls (χ^2 = 4.10, p = 0.044). Likewise, the prevalence of hepatosplenomegaly was significantly higher (43.7%) in children aged 9 – 13 years compared to those aged 6 – 8 years (32.5%) and those aged 3 – 5 years (23.7%) (χ^2 = 25.34, p < 0.001).

4.3.3. Prevalence of *S. haematobium* related pathology

Out of the 1546 children who were examined by ultrasound, 67 children (4.3%) had *S. haematobium* related lesions of the urinary tract. The majority of lesions occurred in the urinary bladder (Table 4.2).

Table 4.2 Prevalence of *S.haematobium* related pathology as detected by ultrasound (n = 1546).

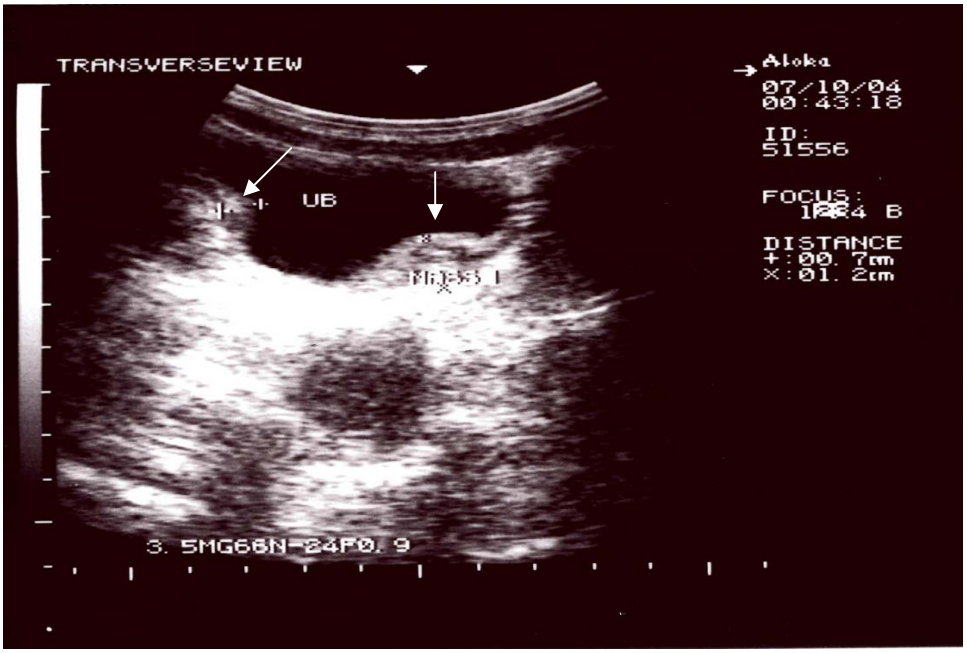
Organ affected	**Number with pathology (%)**		**P-Value**
	Boys (n = 759)	**Girls (n = 787)**	
Urinary bladder	50 (6.6)	13 (1.7)	< 0.001
Kidney/ureters	4 (0.5)	0	NA
Overall pathology	**54 (7.1)**	**13 (1.7)**	**< 0.001**

NA means not applicable

The observed lesions were most frequently mild to moderate and included distortion of bladder shape, focal to diffuse bladder wall irregularity, focal to diffuse bladder wall thickening and single masses/polyps. Overall mild to moderate urinary tract lesions were observed in 59 children (3.8%). Severe lesions including multiple masses/polyps were observed in 5 children (0.3%) and marked dilatation of ureters and/or hydronephrosis were observed in 3 children (0.1%). No cases of urinary bladder wall calcification were detected.

Figure 4.2a Longitudinal scan of the urinary bladder showing thickened inner bladder wall (arrows) due to *S. haematobium* infection.

Figure 4.2b Transverse scan of the urinary bladder showing masses in the inner bladder wall (arrows) due to *S. haematobium* infection.



Pathological changes of the urinary tract were significantly associated with age and sex. The prevalence of urinary bladder pathology was higher (8.4%) in children aged 9 – 13 years compared to those aged 6 – 8 years (4.1%) and those aged 3 – 5 years (0.9%) ($\chi^2 = 19.46$, $p < 0.001$). The prevalence of urinary bladder pathology was significantly higher in boys (7.0%) than girls (1.8) ($\chi^2 = 25.24$, $p < 0.001$). The prevalence of urinary tract pathology differed significantly among schools ($\chi^2 = 19.62$, $p < 0.01$) reflecting variation in transmission and infection intensity of *S. haematobium*. Although it was not consistent schools with the higher prevalence and infection intensity of *S. haematobium* tended to have the highest prevalence of urinary tract pathology (table 4.3).

Table 4.3 Prevalence (n = 1546) of urinary tract (UT) pathology by school in relation to prevalence and infection intensity (expressed as geometric mean parasite count of positive samples only) of *S. haematobium*.

School	Number with UT pathology (%)	S. haematobium prevalence (%)	Geometric mean S. haematobium eggs/10ml urine (95% CI)
Mwamayombo (n = 279)	11 (3.9)	31 (11.1)	16 (9-27)
Nyashimo (n = 302)	2 (0.7)	55 (18.2)	10 (6-15)
Bulima (n = 211)	9 (4.3)	49 (23.2)	17 (10-28)
Milambi (n = 202)	12 (5.9)	65 (33.2)	23 (14-37)
Ihale (n = 255)	10 (3.9)	46 (18.0)	11 (6-18)
Ijitu (n = 297)	23 (7.7)	(19.9)	23 (15-35)
*P-Value	< 0.01	< 0.001	0.037

*** P-Values refer to differences among schools**

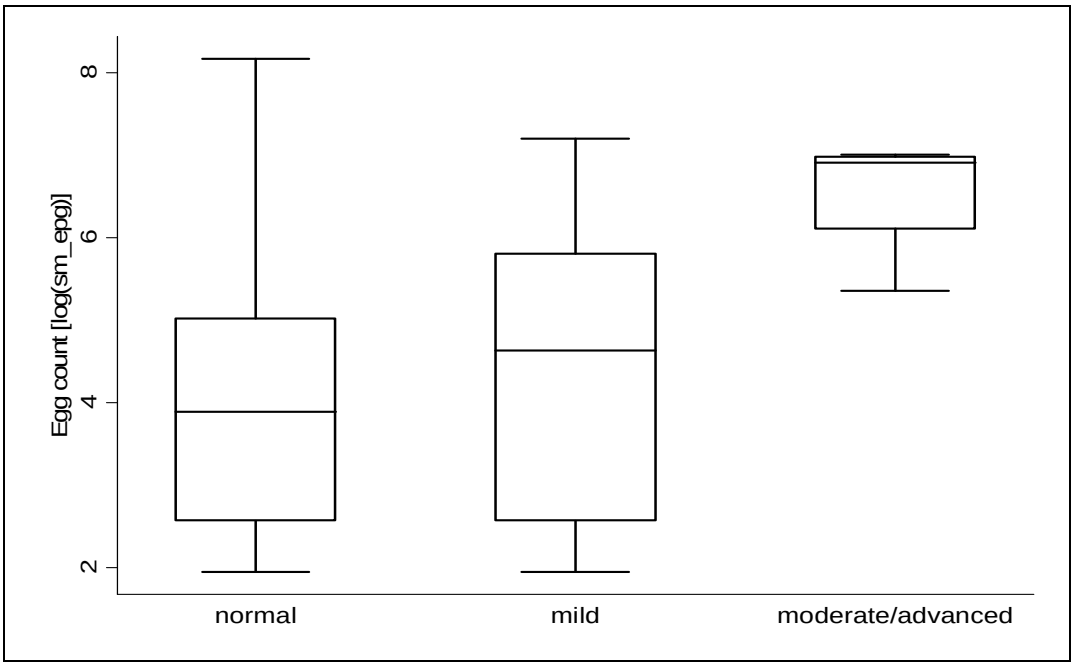
4.3.4. Relationship between liver and spleen pathology and infection status

The prevalence of PPF was significantly higher in children with moderate to heavy *S. mansoni* infection compared to children with light or no *S. mansoni* infection (5.1% vs 1.8% and 1.8%, respectively) (χ^2 = 8.98, p = 0.011). The prevalence of enlargement of the left liver lobe (hepatomegaly) was significantly higher in children with moderate to heavy *S. mansoni* infection compared to children with light or no *S. mansoni* infection (84.3% vs 80.1% and 77.0%, respectively) (χ^2 = 6.84, p = 0.033). The prevalence of splenomegaly was significantly higher in children with high malaria parasite density compared to those with low or no malaria parasites (83.8% vs 59.3% and 30.9%, respectively (χ^2 = 133.47, p < 0.001). Furthermore the prevalence of splenomegaly was significantly higher in children with hookworm infection compared to children without hookworm infection (53.5% vs 37.4%) (χ^2 = 22.32, p < 0.001).

The prevalence of hepatosplenomegaly was 75.7% in children with high malaria parasite density compared to 47.8% and 24.9% in children with low and no malaria parasites, respectively (χ^2 = 105.44, p < 0.001). Hepatosplenomegaly was also significantly more prevalent in hookworm infected children compared to uninfected children (45.3% vs 29.9%) (χ^2 = 22.34, p < 0.001). Enlargement of the portal vein diameter (PVD) which is an indicator of increased portal pressure was significantly more

prevalent in children with high malaria parasite density compared to children with low or no malaria parasites (35.1% vs 16.1% and 14.2%, respectively) ($\chi^2 = 12.53$, $p < 0.01$). The prevalence of enlarged PVD was also associated with area of residence (school) ($\chi^2 = 21.99$, $p = 0.015$) but not with *S. mansoni* infection ($\chi^2 = 0.165$, $p = 0.921$).

Figure 4.3 Box and whisker plot* showing the relationship between median and range of log transformed *S. mansoni* egg counts (Log(sm epg)) and levels of PPF expressed as normal (n = 1511), mild (n = 29) and moderate/advanced (n = 6).



*** The horizontal bars within each box represents the median egg count while the lower and upper edge of each box indicates the 25th and 75th percentiles, respectively and the whiskers represents the minimum and maximum values (range). Normal; n =1511. Mild; n = 29. Moderate/advanced; n = 6**

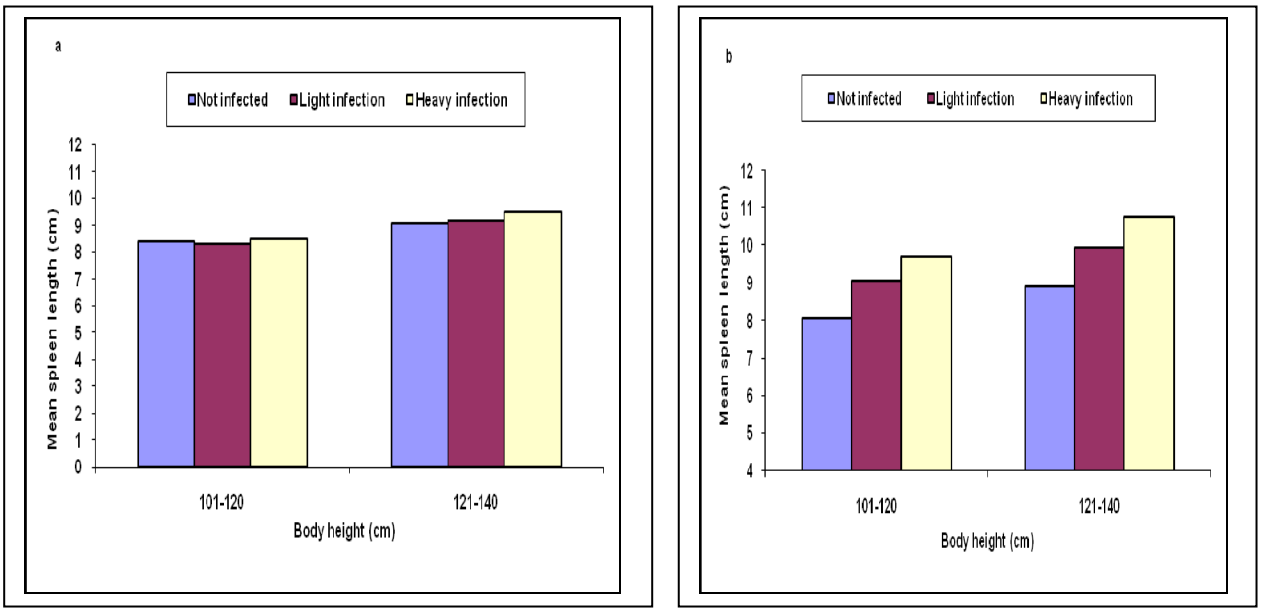
Children with moderate to advanced PPF had significantly higher *S. mansoni* egg counts compared to children with mild or no PPF ($F = 7.16$, $p < 0.001$) (Fig. 4.3).

4.3.5. Relationship between liver size, spleen size, PVD and single and multiple parasite infections

To determine the relationship between organ pathology and single and multiple parasite infections, height adjusted size of the left liver lobe (liver PSL in cm), spleen (spleen length in cm) and portal vein diameter (PVD in mm) were compared according to infection status. Height adjustment was performed according to four height groups i.e. 80-100cm (n = 51), 101-120cm (n = 866), 121-140cm (n = 622) and 141-160cm (n = 7). Children within height groups 80-100cm and 141-160cm were excluded from further analysis because they were very few. *S. mansoni* infection intensity had a significant effect on size of the left liver lobe (liver PSL) ($F = 19.02$, $p < 0.001$). For each height group, children with

moderate to heavy *S. mansoni* infection had significantly larger left liver lobe compared to children with light or no *S. mansoni* infection. *P. falciparum* infection only or in combination with *S. mansoni* had no significant effect on the size of the left liver lobe ($p > 0.05$).

Figure 4.4 Relationship between size of the spleen (spleen length in cm) and *S. mansoni* infection (a), *P. falciparum* infection (b) or both (c) (n = 1488).



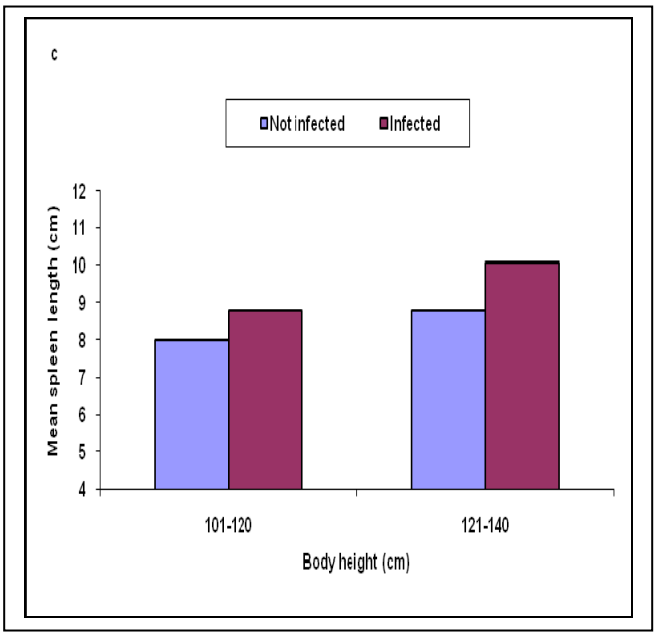
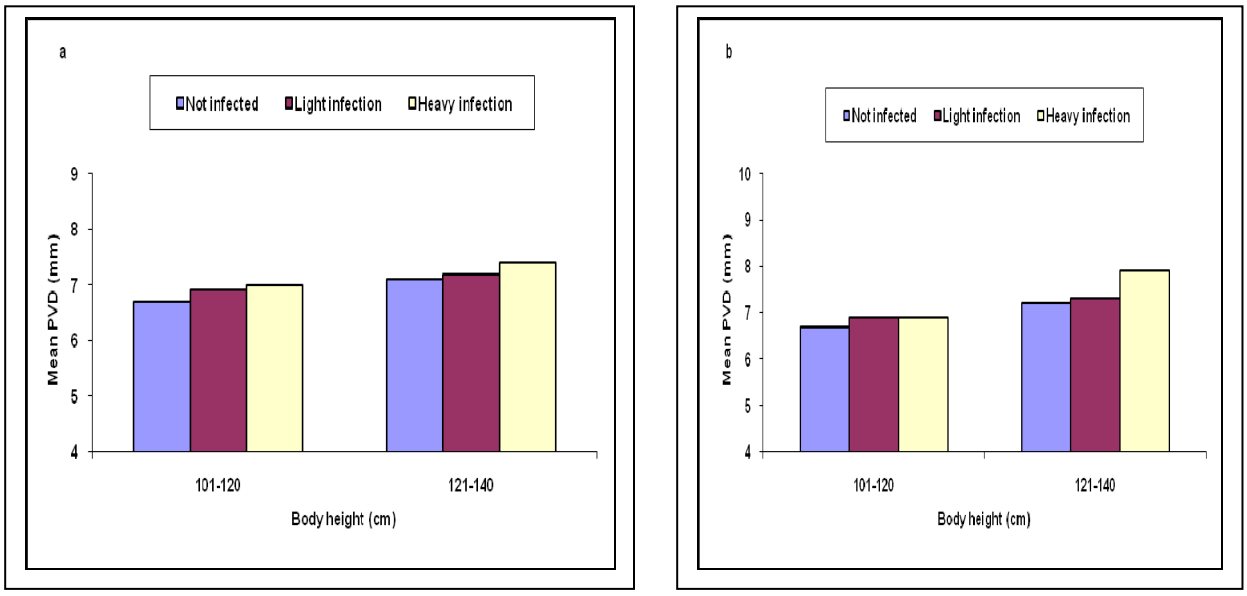


Figure 4.4b shows that *P. falciparum* infection had a significant effect on the size of the spleen. For each height group, children with heavy *P. falciparum* infection had significantly larger spleens compared to children with light or no *P. falciparum* infection ($p < 0.001$). Children concurrently infected with *P. falciparum* and *S. mansoni* (figure 4.5c) had significantly larger spleens compared to uninfected children ($p < 0.05$).

Figure 4.5 Relationship between size of the portal vein diameter (PVD in cm) and *S. mansoni* infection (a), *P. falciparum infection* (b) or both (c) (n = 1488).



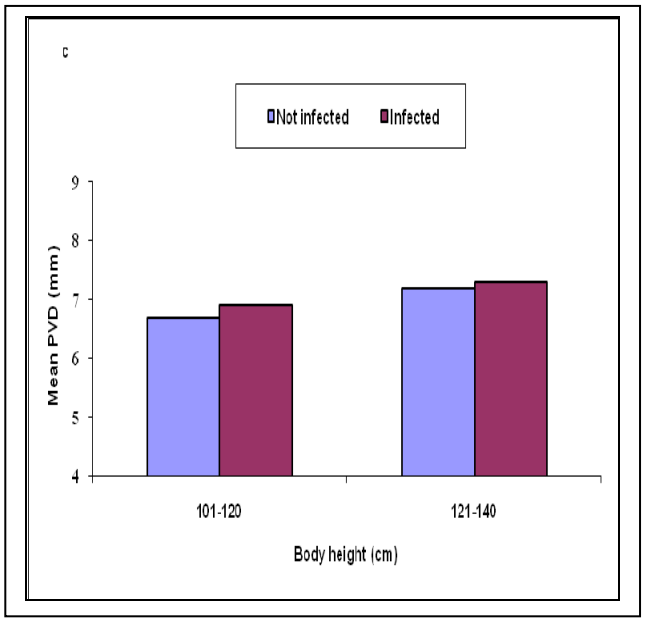


Figure 4.5a shows that *S. mansoni* infection had a significant effect on the size of the portal vein diameter (PVD). For each height group, children with moderate to heavy *S. mansoni* infection had significantly larger portal vein diameters compared to children with light or no *S. mansoni* infection ($p < 0.05$). *P. falciparum* infection only (figure 4.5b) or in combination with *S. mansoni* (figure 4.5c) did not have an effect on the size of PVD ($p > 0.05$).

Table 4.4 Relationship between height adjusted size of left liver lobe(liver PSL in cm), size of the spleen (spleen length in cm) and portal vein diameter (PVD in mm) and multiple parasite infections (Figures in brackets indicates 95% confidence intervals of the mean) (n = 1488).

Organ size/Infection status	**Height group (cm)**	
	101 - 120	121 - 140
Liver psl (cm)		
Not infected	7.4 (7.3-7.5)	7.8 (7.6-8.0)
Singe parasite	7.5 (7.4-7.6)	8.0 (7.9-8.1)
Two or more parasites	7.8 (7.5-8.0)	8.1 (8.0-8.3)
P-Value	< 0.01	0.056
Spleen length (cm)		
Not infected	7.9 (7.8-8.0)	8.6 (8.4-8.9)
Singe parasite	8.5 (8.3-8.6)	9.2 (9.0-9.4)
Two or more parasites	8.9 (8.6-9.1)	9.4 (9.3-9.6)
P-Value	< 0.001	< 0.001
PVD (mm)		
Not infected	6.5 (6.4-6.7)	6.9 (6.7-7.1)
Singe parasite	6.7 (6.6-6.9)	7.2 (7.0-7.3)
Two or more parasites	7.0 (6.8-7.1)	7.4 (7.2-7.6)
P-Value	< 0.001	< 0.001

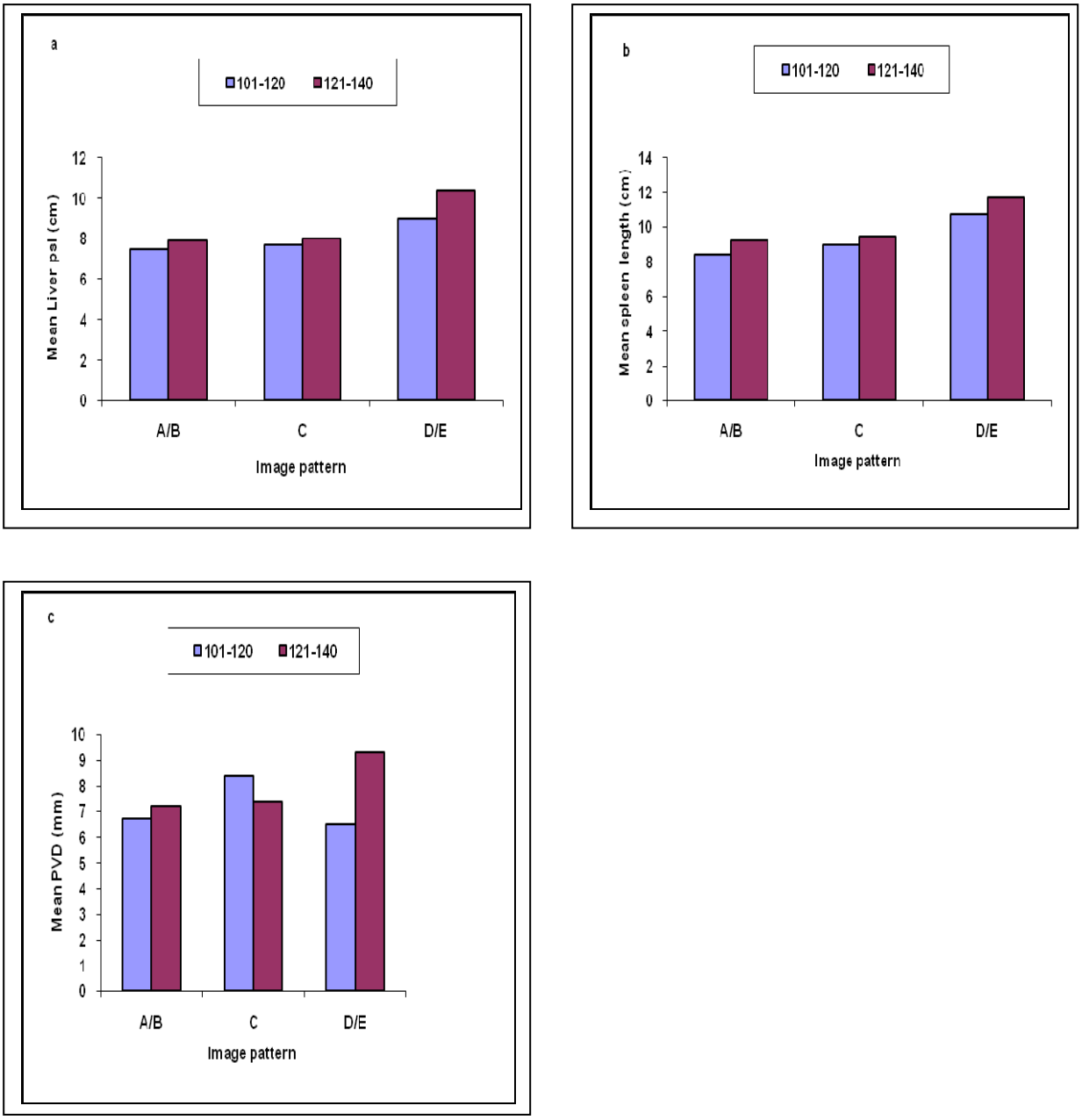
Overall table 4.4 shows that for liver, spleen and portal vein, organ size increased with increasing number of parasite infections. Children with two or more parasite infections had significantly larger organs compared to children without any parasite infection or those with single parasite infections.

4.3.6. Relationship between liver image pattern and liver size, spleen size and PVD

Figure 4.6 shows the relationship between liver image pattern and size of the left liver lobe (liver psl in cm) (a), spleen size (spleen length in cm) (b) and portal vein diameter (PVD in mm) (c) stratified by

body height groups. Children within height groups 80-100cm and 141-160cm were excluded from this analysis because they were very few.

Figure 4.6 Relationship between liver image pattern and size of the left liver lobe (liver psl in cm) (a), spleen size (spleen length in cm) (b) and portal vein diameter (PVD in mm) (c) stratified by body height groups.



Liver image pattern was categorised as normal (A/B) (n = 1511), mild to moderate (C) (n = 29) and severe/advanced (D/E) (n = 6).

The size of the left liver lobe (mean liver psl) increased gradually with increasing severity of PPF from liver IP A and B to D and E but with significant differences for children in the height group of 121-140cm only (Figure 4.6a). The same pattern was observed for spleen length and PVD (Figure 4.6b and Figure 4.6c, respectively) where significant differences were observed for all height groups (except for PVD in the height group of 101-120cm). Overal children with moderate to severe PPF (liver IP D - E) had significantly enlarged left liver lobe, spleen and portal vein diameter compared to children with mild or without PPF (liver IP A – C) ($p < 0.05$).

4.3.7. Predictors of liver and spleen pathology

Age group and *S. mansoni* infection intensity were significant predictors of PPF whereas school was not. Older children (6 years and above) were about 10 times more likely to develop PPF compared to younger children (3 – 5 years). Likewise, children with moderate to heavy *S. mansoni* infection were more than two times likely to develop PPF compared to children with light or no *S. mansoni* infection (Table 4.5).

Table 4.5 Multivariate logistic regression analysis showing predictors of PPF (n = 1546).

Independent variable	**Categories**	**Adjusted Odds Ratio (95% CI)**	**P-value**
Age group	3 – 5 years	1	
	6 – 8 years	9.10 (1.231 - 67.358)	0.031
	9 – 13 years	11.450 (1.428 - 92.552)	0.022
S. mansoni infection intensity			
	Not infected	1	
	Light infection	0.857 (0.350 - 2.098)	0.736
	Moderate/heavy infection	2.356 (1.064 – 5.216)	< 0.034
School	Mwamayombo	1	
	Nyashimo	0.293 (0.076 – 1.120)	0.073
	Bulima	0.232 (0.047 – 1.130)	0.071
	Milambi	0.294 (0.061 – 1.140)	0.126
	Ihale	0.998 (0.372 – 2.678)	0.997
	Ijitu	1.110 (0.434 – 2.835)	0.829

Table 4.6 Multivariate logistic regression analysis showing predictors of enlarged left liver lobe (n = 1546).

Independent variable	Categories	Adjusted Odds Ratio (95% CI)	P-value
S. mansoni infection intensity	Not infected	1	
	Light infection	1.225 (0.91 – 1.656)	0.187
	Moderate/heavy infection	1.620 (1.061 – 2.450)	0.025
School	Mwamayombo	1	
	Nyashimo	1.521 (1.00 – 2.31)	0.048
	Bulima	1.170 (0.754 – 1.817)	0.483
	Milambi	1.363 (0.867 – 2.581)	0.179
	Ihale	1.053 (0.701 – 1.581)	0.804
	Ijitu	0.934 (0.637 – 1.371)	0.729

Only *S. mansoni* infection and school were predictors of enlarged left liver lobe (as assessed by ultrasound) after adjusting for age and sex. Children with moderate to heavy infection of *S. mansoni* were more likely to have enlarged left liver lobe compared to children with light or no *S. mansoni infection*. Likewise, children attending Nyashimo primary school were more likely to have enlarged left liver lobe compared to children attending other schools (table 4.6).

Table 4.7 Multivariate logistic regression analysis showing predictors of splenomegaly in school and pre-school children in Magu district, Tanzania (n = 1546).

Independent variable	Categories	Adjusted Odds Ratio (95% CI)	P-value
Age group	3 – 5 years	1	
	6 – 8 years	1.785 (1.358 – 2.346)	< 0.001
	9 – 13 years	3.480 (2.447 – 4.950)	< 0.001
Sex	Male	1	
	Female	0.80 (0.649 – 0.982)	0.033
Malaria parasite density	Not infected	1	
	Low	3.230 (2.546 – 4.093)	< 0.001
	High	13. 549 (5.532 – 33.176)	< 0.001
Hookworm infection	Not infected	1	
	Light infection	1.763 (1.317 – 2.360)	< 0.001

As seen in table 4.7 age group, malaria parasite density and hookworm infection were significant predictors of enlarged spleen (splenomegaly) as assessed by ultrasound. *S. Mansoni* infection intensity was not a predictor of splenomegaly ($p > 0.05$).

Table 4.8 Multivariate logistic regression analysis showing predictors of hepatosplenomegaly in school and pre-school children in Magu district, Tanzania (n = 1546).

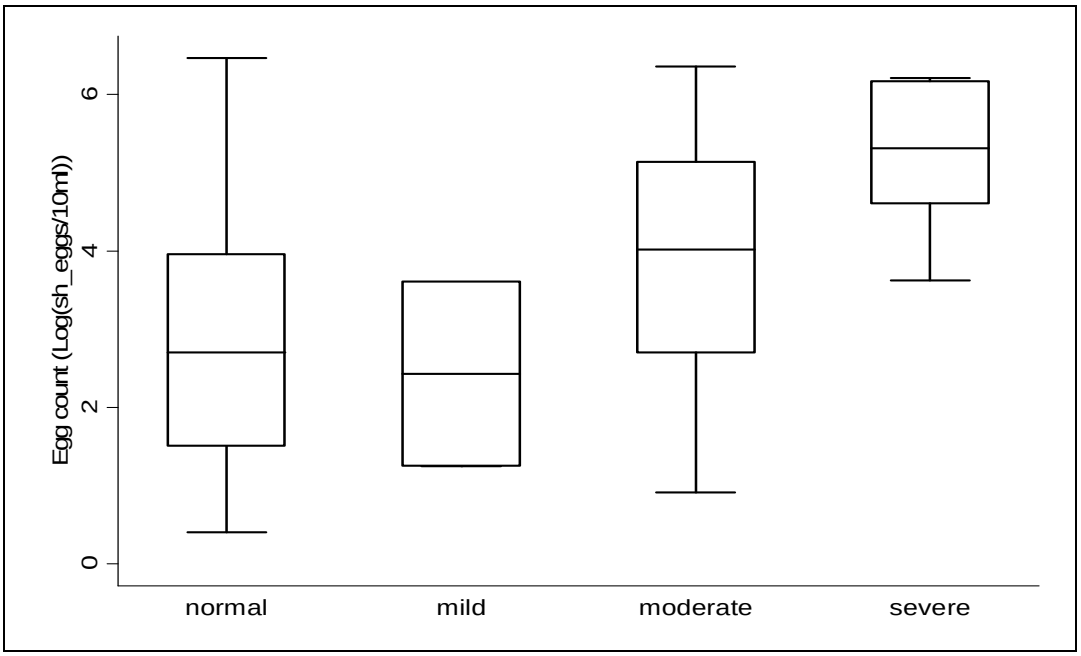
Independent variable	**Categories**	**Adjusted Odds Ratio (95% CI)**	**P-value**
Age group	3 – 5 years	1	
	6 – 8 years	1.552 (1.166 – 2.066)	< 0.01
	9 – 13 years	2.489 (1.734 – 3.568)	< 0.001
Malaria parasite density	Not infected	1	
	Low	2.696 (2.126 – 3.419)	< 0.001
	High	10.294 (4.757 – 22.276)	< 0.001
Hookworm infection	Not infected	1	
	Light infection	1.847 (1.394 – 2.448)	0.001

Malaria parasite density and hookworm infection were significant predictors for enlargement of both the liver and spleen (hepatosplenomegaly) (as assessed by ultrasound) after adjusting for age and sex. *S. mansoni* infection was not a significant predictor of hepatosplenomegaly in the multivariate analysis though the prevalence of hepatosplenomegaly tended to increase with increasing infection intensity of *S. mansoni*. The prevalence of hepatosplenomegaly was 37.8% in children with moderate to heavy *S. mansoni infection* compared to 30.3% and 31.9% in children with light and no *S. mansoni* infection, respectively ($\chi^2 = 3.763$, $p = 0.152$).

4.3.8. Relationship between urinary tract pathology and infection status

The prevalence and severity of urinary tract pathology was dependant on intensity of *S. haematobium* infection. The prevalence of pathology was significantly higher in children with heavy *S. haematobium* infection compared to children with light or no *S. haematobium* infection (27.7% vs 9.5% and 1.7%, respectively ($\chi^2 = 157.72$, $p < 0.001$).

Figure 4.7 Box and whisker plot* showing the relationship between median and range of *S. haematobium* egg count (Log(sh_eggs/10ml)) and severity of urinary tract pathology expressed as



*** The horizontal bars within each box represents the median egg count while the lower and upper edge of each box indicates the 25th and 75th percentiles, respectively and the whiskers represents the minimum and maximum values (range). Normal (n = 1480), mild (n = 9), moderate/severe (n = 59).**

Children with moderate to severe urinary tract pathology had significantly higher *S. haematobium* egg counts compared to children with mild or no urinary tract pathology ($F = 9.85$, $p < 0.001$) (Figure 4.7).

4.3.9 Predictors of urinary tract pathology

Table 4.9 Multivariate logistic regression analysis showing predictors of urinary tract (UT) pathology (n = 1546).

Independent variable	Categories	Adjusted Odds Ratio (95% CI)	P-value
Age	3 – 5 years	1	
	6 – 8 years	3.764 (1.336 – 10.610)	0.012
	9 – 13 years	7.965 (2.684 – 23.644)	< 0.001
Sex	Male	1	
	Female	0.242 (0.132 – 0.440)	< 0.001
S. haematobium infection intensity	Not infected	1	
	Light infection	5.230 (2.746 – 9.960)	< 0.001
	Moderate/heavy infection	20.934 (10.931 – 40.10)	< 0.001
S. mansoni infection intensity	Not infected	1	
	Light infection	1.160 (0.676 – 1.988)	0.591
	Moderate/heavy infection	0.193 (0.058 – 0.640)	< 0.01
Hookworm infection	Not infected	1	
	Light infection	3.450 (2.035 – 5.850)	< 0.001
School	Mwamayombo	1	
	Nyashimo	0.141 (0.031 – 0.645)	0.012
	Bulima	0.732 (0.286 – 1.872)	0.515
	Milambi	1.300 (0.551 – 3.067)	0.549
	Ihale	0.776 (0.316 – 1.910)	0.581
	Ijitu	1.650 (0.773 – 3.517)	0.196

Age, sex, and infection intensity of *S. haematobium* were significant predictors of urinary tract pathology. Moderate to heavy *S. mansoni* infection was reduced the risk for urinary tract pathology after adjusting for age and sex. Nyashimo primary school also led to a reduced risk for urinary tract pathology (table 4.9).

4.4. Discussion

This study reports comprehensively on clinical and ultrasonographical findings of morbidity related to *S. mansoni* and *S. haematobium* infections in school and preschool children in the lake zone of Tanzania. This area is also endemic for malaria with a prevalence of 29.8% found at the baseline survey. Previous studies (Malenganisho *et al*, 2008; Kardorff *et al*, 1997) described morbidity patterns due to *S. mansoni* infection mainly in adults. Findings of this study show that though the prevalence of PPF was low, it was detected in children as young as 5 years of age. This finding combined with observed enlargement of both the liver and spleen indicates that schistosomiasis related hepatosplenic disease is common in this area and develops at a relatively young age as it have been established by other studies (Boisier *et al*, 2001, Kardorf *et al*, 1997; Booth *et al*, 2004b; Malenganisho *et al*, 2008). Kardorf *et al* (1997) observed a prevalence of PPF of up to 13.3% in children aged 10 years or less in a nearby district of Ukerewe, an island in Lake Victoria. The prevalence of PPF, hepatomegaly, splenomegaly and increased portal vein diameter tended to be higher in boys than girl, a finding which was also reported by Kardoff *et al* (1997) and is explained by differences in infection levels which in turn can be explained by different water contact patterns. Further, the prevalence of PPF was low compared to ultrasound detected enlargement of liver, spleen and PVD, suggesting that most children had hepatosplenic disease without PPF, a finding which is in line with findings of previous studies (Gryseels and Polderman, 1987; Boisier *et al*, 2001; Vennervald *et al*, 2004). The prevalence of PPF and size of the left liver lobe correlated well with *S. mansoni* egg counts indicating the role of *S. mansoni* infection in the development of PPF and enlargement of the liver.

Ultrasound examination detected more cases of organomegaly compared to clinical examination. This could be a result of difference in sensitivity of the two methods as ultrasound examination would be expected to detect smaller changes in organ size compared to physical palpation as was also reported by other studies (Homeida *et al*, 1988; Richter *et al*, 1992; Tomayo *et al*, 1993; Kardoff *et al*, 1997 and Vennervald *et al*, 2004a). While ultrasound technique is known to be a highly sensitive, precise and standardized technique (Homeida *et al*, 1988; Doehring-Schwerdtfeger *et al*, 1989; Richter, 1992; Vennervald and Dunne, 2004b), physical palpation is a les sensitive and observer dependant technique (Tomayo *et al*, 1993). Other explanations for this could be the possibility that most enlarged organs were of soft consistence which makes it difficult to be detected clinically. Furthermore, the suitability of Niamey protocol (Richter *et al*, 2000) for assessing organomegaly by adjusting organ enlargement measurements for height based on the Senegalese normogram can be questionned. By using the cut of values provided based on standard deviations in organometric measurements observed in a healthy uninfected Senegalese population (Yazdanpanah *et al*, 1997), it is very likely that the prevalence of organ enlargement in the current study was overestimated as has been observed by previous studies (King *et al*, 2003; Booth *et al*, 2004b; Malenganisho *et al*, 2008).

Contrary to what was observed by studies of Malenganisho *et al* (2008), Vennervald *et al* (2004a) and Homeida *et al* (1988), enlargement of the liver, spleen and PVD correlated well with the severity of PPF. The highest values of left liver lobe, spleen length and PVD were observed in children with liver IP D and E indicating the importance *S. mansoni* infection as the aetiology of both PPF and other types

of organ pathology. The lack of correlation between severity of PPF and other organometric parameters in the studies mentioned above may be as a result of many factors including variation in disease progression leeding to PPF and organ enlargement (Chan *et al*, 1996; Ouma *et al*, 2001; Lambertucci *et al*, 2000; Abdel-Wahab *et al*, 1989), age distribution and *S. mansoni* transmission intensity in the studied populations (Gryseels and Polderman, 1987), nutritional and genetic confounders (Cobertt *et al*, 1992; Secor *et al*, 1996; Dessein *et al*, 1999). Variation in disease progression can occur at individual and community level and may be affected by physiological, immunological, genetic and environmental factors (Vennervald *et al*, 1998). In the study of Vennervald *et al* (2004a), the occurrence of organ enlargement in the absence of ultrasound detectable PPF was attributed to the possibility that inflammatory and granulomatous changes in the walls of small portal branches might have lead to increased portal pressure, splenomegaly and dilation of portal vein in the absence of ultrasound detectable PPF. An altenative explanation for the absence of correlation between PPF and organ enlargement could be that while ultrasonoghraphy is more sentitive in detecting organ enlargement, it is not sensitive enough to detect and correctly classify all cases of early mild degrees of PPF (Vennervald *et al*, 1998).

Although the prevalence and infection intensity of *S. mansoni* infection among schools varied at baseline (see chapter 3), these variations were not reflected in the prevalence of PPF as there was no differences in prevalence of PPF between schools. This observation could be explained by the fact that there were few children with ultrasound detectable PPF compared to egg positive children. This in turn could be explained by the relatively low age distribution of children studied and hence short duration of exposure to *S. mansoni* infection. About 60% of children studied were in the age group of 6-8 years which means that they had not been exposed for a period long enough for liver pathology to develop. This finding concurs with findings of Booth *et al* (2004b) in two adjacent communities in Uganda where the two communities differed significantly with regards to the prevalence of PPF but were comparable in terms of *S. mansoni* infection intensity. The authors attributed this finding to differences in duration of residence by community members in the two communities and hence differences in duration of exposure to *S. mansoni* infection. Supprisingly, portal systemic collaterals were detected in one 7 year old boy who had both liver, spleen and portal vein diameter grossly enlarged. The child was anaemic and had moderate *S. mansoni* infection and advanced/severe PPF (liver IP E). He was negative for malaria, *S. haematobium* and STH infection. Portal systemic collaterals are a severe manifestation of increased portal pressure due to *S. mansoni* related PPF particularly in adults. Apart from *S. mansoni* infection, it can also be caused by liver cirrhosis, viral hepatitis, liver abscesses, liver neoplasms and excessive acohol consumptions (Pereira *et al*, 1994; Mabrouk, 1997; Malenganisho *et al,* 2008). However, in this case, the presence of a combination of enlargement of the liver, spleen and portal vein diameter in the presence of moderate *S. mansoni* infection (epg 210) and advanced PPF is a strong indicator that the observed portal systemic collaterals were due to increased portal pressure associated with pathology due to *S. mansoni* infection. This shows a clear evidence that under conditions of high transmission, severe *S. mansoni* related pathology can develop at a relatively young age.

P. falciparum infection did not have a significant effect on size of the left liver lobe although the prevalence of enlarged left liver lobe tended to increase with increasing malaria parasite density. These result tend to agree with findings of Wilson *et al. (*2007) who observed that malaria parasitaemia was not associated with hepatomegaly in Kenyan children. However, chronic exposure to *P. falciparum* infection expressed in terms of high levels of *P. falciparum* specific IgG3 antibodies was associated

with both hepatomegaly and splenomegaly. Combining these findings leads to a conclusion that acute *P. falciparum* infection with low level parasitaemia had no effect on liver enlargement. However, chronic *P. falciparum* infections was associated with liver enlargement. Likewise, acute *P. falciparum* infection with high malaria parasite density (≥ 5000 parasites/µl of blood) was also associated with liver enlargement. While splenomegaly was not associated with *S. mansoni* infection, it was associated with both the prevalence and intensity of *P. falcipatum* infection suggesting *P. falciparum* infection was the major factor responsible for enlargement of the spleen as it has been reported by other studies (Vennervald *et al* 2004; Tschikuka *et al*, 1996; Booth *et al*, 2004a; Wilson *et al*, 2007).

The clinical picture of the eight children who were diagnosed with hepatomegaly indicated that *S. mansoni* and *P. falciparum* infections are major causative agents for hepatosplenic disease in the studied population. Six (75%) of these children had both *S. mansoni* and *P. falciparum* infections and were anaemic. Similarly, all children who had enlargement of both the liver and spleen (hepatosplenomegaly) had patent *S. mansoni* and *P. falciparum* infections and were anaemic. These observations are in accordance with reports by other studies (Corbett *et al*, 1992; Wilson *et al.* 2007). The mechanisms underlying liver and spleen involvement in *S. mansoni* and *P. falciparum* infections has been described in detail in chapter 1. Briefly, for *P. falciparum* infection, pathological effects to the host are primarily observed during the erythrocytic phase of infection. During this phase, there is extensive destruction of infected erythrocytes and release of parasite and erythrocyte materials into host circulation. Contents of destroyed red blood cells that are released into bloodstream stimulate release of tumor necrosis factor (TNF) and other cytokines which in turn stimulate inflammatory and immune responses against parasite antigens. Systemic complications develop including altered body physiology and biochemistry, anaemia and tissue and organ hypoxia (Day *et al*, 1999; Cranston, 1966). The inflammatory and immune processes described above inevitably involve the liver and spleen which become enlarged (Warrel *et al* 2002). For *S. mansoni* infections, tissue damage result from host immune reaction against schistosome eggs deposited in host tissues. This reaction leads to granuloma formation in gastro-intestinal organs including the liver and spleen (Davis, 2009). Chronic *S. mansoni* infection thus results in periportal fibrosis, portal hypertension and esophageal varices and enlargement of the liver and spleen (Warren, 1987; Davis, 2009)**.**

In multiple logistic regression analysis it was demonstrated that children attending three schools namely Nyashimo, Bulima and Milambi had a reduced risk of developing PPF. This finding may reflect existence of variations in exposure and transmission patterns of *S. mansoni* between schools which is supported by the parasitological results (chapter 3) which shows marked variation in prevalence and infection intensity of *S. mansoni* among study schools. This observation is also supporter by findings of other studies (Boisier *et al*, 2001; Vennervald et al 2004a; Booth et al, 2004a). Variations in the level of exposure and transmission of *S. mansoni* and other parasitic infections and their related morbidity can occur even within very small geographical areas and are caused by a number of factors including distance to transmission sites, microclimate and ecology of vectors and intermediate hosts (Booth *et al*, 2004; Klumpp and Webbe, 1987; Kloos *et al*, 1997).

One interesting finding of this study is the demonstration of a significant effect of hookworm infection on splenomegaly, hepatosplenomegaly and urinary tract pathology. This observation was supported by baseline parasitological data (not shown) which demonstrated that hookworm infection was significantly associated with both *S. mansoni* and *S. haematobium* infections whereby hookworm

infected children had significantly higher prevalence of *S. mansoni* and *S. haematobium* infections compared to uninfected children. Further, hookworm infected children had higher infection intensities of *S. mansoni* compared to uninfected children. On the other hand, *S. mansoni* infection was positively associated with the prevalence of hookworm infection. One possible explanation for this observation could be that hookworm infection has an intensity dependant synergistic effect on host immune responses against *S. mansoni* and *S. haematobium* infections. Since development of schistosome related pathology is dependent on host immune response against schistosome eggs, any factor that will influence host immune responses against schistosome infection will also influence the level of schistosome related pathology. The observation of a positive association between hookworm infection and other helmnth species was also reported in Brazil (Fleming *et al* 2006) and in Cote D'Ivoire (Kaiser *et al*, 2002). In the Brazil study it was observed that hookworm infection had a significant positive association with *S. mansoni* and *A. lumbricoides* infections. The study observed that individuals coinfected with either hookworm and *S. mansoni* or hookworm and *A. lumbricoides* had significantly higher infection intensities (mean epg) than individuals infected with either parasite. In the study from Cote D'Ivore it was found that increasing infection intensity of *S. mansoni* was significantly correlated with an increased likelihood of concomitant hookworm infections. These observations are also in line with immunological studies that demonstrated cross reactivity between *S. mansoni* specific antibodies and hookworm (*N. americanus*) antibodies (Pritchard *et al*.1991; Correˆa-Oliveira *et al*, 1988, 2002; Timothy *et al*, 1992). In line with cross-reactivity between *S. mansoni* and hookworm or other STH specific antibodies (which means that these parasites share common antigens) is the possibility of existence of common immune evarsion mechanisms for example by secretion of immunomodulatory molecules by one or both prasites which favour survival of both parasites in cocurently infected hosts. This view is in line with findings of studies which observed that helminth infections have the ability to secrete immunomodulation molecules which enable these parasites to escape host immune responses (Geiger, 2008; Quinnell *et al*, 2004a; Chow *et al*, 2000; Loukas and Prociv, 2001; Hsieh *et al*, 2004; Maizels *et al*, 2004). Immunological mechanisms such as the differential activation of type type 2 T helper cell subsets by helminth infections may partly explain the observed association between hookworm infection and *S. mansoni* and *S. haematobium* related pathology as both hookworm, *S. manasoni* and *S. haematobium* infections are characterized by immune responses dominated by type 2 T helper cells (Quinnell *et al*. 2004; Davis, 2009). Besides of involvement of immunological mechanisms as the cause of the observed schistosome-STH interactions, other factors such as favourable environmental conditions for development and survival of parasites involved, low socio-economic status and poor hygienic standards of affected populations, host and parasite genetics and individual predisposition may play a role and hence cannot be completely ruled out (De Silva *et al*, 2003; Utzinger *et al*, 2003; Thapar and Sanderson, 2004; Bethony *et al*, 2006).

Morbidity due to *S. haematobium* infection occurred mainly in the lower urinary tract and was age and sex dependant as has been reported by other studies (Sacko, 2006; Hartz *et al*, 1998; Traore *et al*, 1998). Higher prevalence of urinary tract pathology was observed in boys than girls and in older children than young ones reflecting the age and sex distribution of *S. haematobium* infection in the studied population as has already been described in chapter 3. The prevalence and severity of pathological lesions were associated with intensity of infection of *S. haematobium* indicating that *S. haematobium* infection intensity was an important predictor of urinary tract morbidity consistent with reports of other studies (Sacko, 2006; Mwanje, 1999). The prevalence of urinary tract pathology differed significantly among schools reflecting variation in prevalence and infection intensity of *S.*

haematobium infection among schools as has already been described in chapter 3. Predictors of urinary tract pathology were age, sex, school (as expected), *S. haematobium* infection intensity, hookworm infection and *S. mansoni* infection intensity. Moderate to heavy *S. mansoni* infection was shown to be protective against *S. haematobium* related pathology which could be explained by the inverse distribution of *S. mansoni* and *S. haematobium* in the area. *S. mansoni* is more prevalent near the shoreline whereas *S. haematobium* is more prevalent in the hinterland away from the shoreline (Lwambo *et al*, 1999). School was shown to be significantly associated with urinary tract pathology. Ijitu primary school had one of the highest infection intensity of *S. haematobium* and consequently children at this school had the highest risk of developing urinary tract pathology compared to children attending other schools. On the other hand, Nyashimo primary school had the lowest infection intensity of *S. haematobium* and therefore children at this school had the lowest risk of developing urinary tract pathology.

In conclusion, this study demonstrated that schistosome related morbidity of the liver, spleen and urinary tract is common in school children in the study area even at a very young age and is positively correlated with both the prevalence and infection intensity of *S. mansoni* and *S. haematobium*. For *S. mansoni* infection, variations existed among schools in prevalence and infection intensity which were not reflected in the prevalence of PPF indicating that development of PPF may not necessarily depend on prevalence and infection intensity of *S. mansoni* but on duration of exposure to infection. On the other hand, hepatosplenic disease or hepatosplenomegaly depended mainly on infection intensity of *S. mansoni*, malaria or both indicating the importance of the two infections on morbidity in the study area. Further, findings of the current study demonstrate the importance of morbidity assessment using ultrasound as a means of disease surveillance and evaluation of the impact of schistosomiasis control programmes in school children.

4.5. References

Abdel-Wahab and Mahmoud (1987). Schistosomiassis in Egypt. In: Clinical tropical medicine and communiaclble diseases: International practice and research. Vol. 2, No. 2. Bailliere Tindall, London, UK. pp 371-392.

Abdel-Wahab M. F., Esmat G., Milad M., Abdel-Razek S., Strickland G. T. (1989). Characteristic sonographic pattern of schistosomal hepatic fibrosis. *Am J Trop Med Hyg*, **40** (1): 72-6.

Bethony, J., Brooker, S., Albonico, M., Geiger, S. M., Loukas, A., Diemert, D., Hotez, P. J. (2006). Soil-transmitted helminth infections: Ascariasis, trichuriasis and hookworms, *Lancet*, **367:** 1-12.

Booth, M., Vennervald, B. J., Kenty, L., Butterworth, A. E., Kariuki, H. C., Kadzo, H., Amaganga, C., Kimani, G., Mwatha, J. K., Otedo, A., Ouma, J. H., Muchiri, E., Dunne, D. W. (2004a). Micro-geographical variation in exposure to *S. mansoni* and malaria, and exacerbation of splenomegaly in Kenyan School age children. *BMC Infect Dis*, **4:** 13.

Booth, M., Vennervald, B. J., Kabatereine, N. B., Kazibwe, F., Ouma, J. H., Kariuki, C. H., Muchiri, E., Kadzo, H., Ireri, E., Kimani, G., Mwatha, J. K., Dunne, D. W. (2004b). Hepatosplenic morbidity in two neighbouring communities in Uganda with high levels of aschistosoma mansoni infection but very diffrrent durations of residence. *Trans. Roy. Soc. Trop. Med. Hyg*, **98**: 125-136.

Boisier, P., Ramarokoto C. E., Ravoniarimbinina P., Rabarijaona L., Ravaoalimalala, V. E. (2001). Geographic differences in hepatosplenic complications of schistosomiasis mansoni and explanatory factors of morbidity. *Trop Med Int Health,* **6** (9): 699-706.

Butterworth, A. E., Sturrock, R. F., Ouma, J. H., Mbugua, G. G., Fulford, A. J., Kariuki, H. C., Koech, D. (1991). Comparison of different chemotherapy strategies against Schistosoma mansoni in Machakos District, Kenya: effects on human infection and morbidity. *Parasitolo,* **103** (Pt3): 339-355.

Butterworth, A. E., Curry, A. J., Dunne, D. W., Fulford, A. J., Kimani, G., Kariuki, H. C., Klumpp, R., Koech, D., Mbugua, G., Ouma, J. H. (1994). Immunity and morbidity in human schistosomiasis mansoni. *Trop. Geogr. Med,* **46** (4 Spec No): 197-208.

Butterworth, A. E., Dunne, D. W., Fulford, A. J., Ouma, J. H., Sturrock, R. F. (1996). Immunity and morbidity in Schistosoma mansoni infection: quantitative aspects. *Am. J. Trop. Med. Hyg*, **55** (5 Suppl): 109-15.

Cranston, W. I. (1966). Temprerature regulation. *Br. Med. J*, **2**: 69-75.

Chan, M. S., Guyatt, H. L., Bundy, D., Medley, G. E. (1996). Dynamic models of schistosomiasis morbidity. *Am. J. Trop. Med. Hyg*. **55**, 52 – 62.

Chow, S. C., Brown, A., Pritchard, D. (2000). The human hookworm pathogen *N. Americanus* induces apoptosis in T. lymphocytes. *Par Immunol*, **22**: 21-29.

Corbett, E. L., Butterworth, A. E., Fulford, A. J., Ouma, J. H., Sturrock, R. F. (1992). Nutritional status of children with schistosomiasis mansoni in two different areas of Machakos District, Kenya. *Trans. Roy. Soc. Trop. Med. Hyg*, **86** (3): 266-73.

Corrêa-Oliveira, R., Golgher, D.B., Oliveira, G. C., Carvalho, O. S., Massara, C. L., Caldas, I. R., Colley, D. G., Gazzinelli, G. (2002). Infection with *Schistosoma mansoni* correlates with altered immune responses to Ascaris lumbricoides and hookworm. *Acta Trop,* **83**: 123–132.

Davis, A. (2009). Helminthic infections: Schistosomiasis. In: Manson's tropical diseases. 22nd Ed. Sounders. UK. pp 1425- 1460.

Day, N. P., Hien, T. T., Schollaardt, T., Loc, P. P., Chuong, L. V., Chau, T. T., Mai, N. T., Phu, N. H., Sinh, D. X., White, N. J., Ho, M. (1999). The prognostic and pathophysiologic role of pro- and anti-inflamatory cytokines in severe malaria. *J. Infect. Dis*, **180** (4): 1288-1297.

Dessein, A. J., Marquet, S., Henri, S., El Wali, N. E., Hillaire, D., Rodrigues, V., Prata, A., Ali, Q. M., Gharib, B., De Reggi, M., Magzoub, M. M., Saeed, O. K., Abdelhameed, A. A., Abel L. (1999). Infection and disease in human schistosomiasis mansoni are under distinct major gene control, *Microbes Infect*, **1** (7): 561-567.

De Silva, N. R., Brooker, S., Hotez, P. J., Montresor, A., Engels, D., Savioli, L. (2003). Soil transmitted helminth infections: Updating the global picture. *Trends Parasitol*, **19**: 547-551.

Doehring-Schwerdtfeger, E., Mohamed-Ali, G., Abdel-Rahim, I. M., Kardorff, R., Franke, D., Kaiser, C., Elsheikh, M., Ehrich, J. H. (1989). Sonomorphological abnormalities in Sudanese children with *Schistosoma mansoni* infection: A proposed staging-system for field diagnosis of periportal fibrosis. *Am. J. Trop. Med. Hyg*, **41** (1): 63-9.

Fulford, A. J., Mbugua, G. G., Ouma, J. H., Kariuki, H. C., Sturrock, R, F., Butterworth, A. E. (1991). Differences in the rate of hepatosplenomegaly due to *S. mansoni* infection between two areas in Machakos District, Kenya. *Tran. Roy. Soc. Trop. Med. Hyg*. 1991; **85**: 481-488.

Fleming, F. M., Brooker, S., Geiger, S., Caldas, I. R., Correa-Oliveira, R., Hotez, P. J., Bethony, J. M. (2006). Synergistic associations between hookworm and other helminth species in a rural community in Brazil. *Trop. Med. Int. Health*. Vol. II, No. I, 56-64.

Geiger, S. M. (2008). Immunoepidemiology of *Schistosoma mansoni* infections in endemic populations co-infected with soil-transmitted helminths: Present knowledge, challenges and need for further studies. *Acta trop,* **108:** 118-123.

Gryseels, B., Polderman, A. M. (1987). The morbidity of schistosomiasis mansoni in Maniema (Zaire). *Trans. Roy. Soc. Trop. Med. Hyg,* **81** (2): 202-209.

Hartz, C. F., Vennervald, B. J., Nkulila, T. (1998). Evolution of *Schistosoma haematobium* related pathology over 24 months after treatment with praziquantel among school children in Southern Eastern Tanzania. *Am. J. Trop. Med. Hyg,* **59**: 775-781.

Hsieh, G. C. F., Loukas, A., Wahl, A. M. (2004). A secreted protein from the human hookworm *N. Amaricanus* binds selectively to NK cells and induces IFN-γ production. *J Immun,* **173:** 2699-2704.

Homeida, M., Ahmed, S., Dafalla, A., Suliman, S., Eltom, I., Nash, T., Bennett, J. L. (1988). Morbidity associated with *schistosoma mansoni* infection as determined by ultrasound: A study in Gezira, Sudan. *Am. J. Trop. Med. Hyg,* **39** (2): 196-201.

Kaiser, J., N'Goran, E. K., Singer, B. H., Lengeler, C., Tanner, M., Utzinger, J (2002). Association between *Schistosoma mansoni* and hookworm infections among schoolchildren in Coˆ te d'Ivoire. *Acta Trop,* **84:** 31-41.

Kardorff, R., Gabone, RM., Mugashe, C., Obiga, D., Ramarokoto, CE., Mahlert, C., Spannbrucker, N., Günzler, V, Gryseels, B., Ehrich, JH., Doehring, E. (1997). Schiostosoma mansoni related

morbidity on Ukerewe Island, Tanzania: Clinical, ultrasonographical and biochemical parameters. *Trop Med Int Health* 2, 230-239.

King, C. H., Magak, P., Abdel Salaam, E., Ouma, J. H., Kariuki, H. C., Blanton, R. E. (2003). Mesauring morbidity in schistosomiasis mansoni: Relationship between image parttern, portal vein diameter, and portal branch wall thickness in large scale surveys using new WHO coding guidelines for ultarsound in schistosomiasis. *Trop. Med. Int. Health,* **8** (2): 109-117.

Kloos, H., Fulford, A. J., Butterworth, A. E., Sturrock, R. F., Ouma, J. H., Kariuki, H. C., Thiongo, F. W., Dalton, P. R., Klumpp, R. K. (1997). Spatial patterns of human water contact and *Schistosoma mansoni* transmission and infection in four rural areas in Machakos District, Kenya. *Soc. Sci. Med,* **44** (7): 949-968.

Klumpp, R. K., Webbe, G. (1987). Focal, seasonal and behavioural patterns of infection and transmission of *Schistosoma haematobium* in a farming village at the Volta Lake, Ghana. *J Trop Med Hyg.* **90** (5): 265-281.

Lambertucci, J. R. (1993). Schistosomiasis *mansoni*: Pathological and clinical aspects. In: Peter Jordan., Gerald Webbe, Robert F. Sturrock (1993). Human schistosomiasis. (Eds.) CAB International, Wallingford, UK. 195-235.

Lambertucci, J. R., Serufo, J. C., Gerspacher-Lara, R., Rayes, A. A. M., Teixeira, R., Noble, V., Antunes, C. M. F. (2000). *Schistosoma mansoni* assessment of morbidity before and after control. *Acta Trop*, **77:** 101-109.

Loukas, A and Prociv, P. (2001). Immune responses in hookworm infections. *Clin Microbiol Review,* **14***:* 689-703.

Lwambo, N. J. S., Siza, J. E., Brooker, S., Bundy, D. A. P., Guyatt, H. (1999). Patterns of concurrent hookworm infections and schistosomiasis in school children in Tanzania. *Trans. Roy. Soc. Trop. Med. Hyg,* **93:** 497-502.

Mabrouk, G. M. (1997). Prevalence of hepatitis C infection and schistosomiasis in Egyptian patients with hepatocellular carcinomas. *Dis Markers,* **13** (3): 177-182.

Maizels, R. M., Balic, A., Gomez-Escobar, N., Nair, M., Taylor, M. D., Allen, J. E. (2004). Helminth parasites-masters of regulation, *Immunol. Rev,* **201:** 89-116.

Malenganisho, W. L., Magnussen, P., Friis, H., Siza, J., Kaatano, G., Temu, M., Vennervald, B. J. (2008). *Schistosoma mansoni* morbidity among adults in two villages along Lake Victoria shores in Mwanza District, Tanzania. *Trans. Roy. Soc. Trop. Med. Hyg*, **102** (6): 532-541.

Mwanje, M. T. (1999). Infection pattern and morbidity in urinary schistosomiasis (*schistosoma haematobium*) in Mwamanga village, Kwale district, Kenya. PhD thesis, Institute of Zoology, Faculty of Natural Sciences, University of Copenhagen, Denmark.

Mwatha, J. K., Jones, F. M., Mohamed, G., Naus, C. W., Riley, E. M., Butterworth, A. E., Kimani G., Kariuki, C. H., Ouma, J. H., Koech, D., Dunne, D. W. (2003). Associations between anti-*Schistosoma mansoni* and anti-*Plasmodium falciparum* antibody responses and hepatosplenomegaly, in Kenyan schoolchildren. *J. Infect. Dis*, **187** (8):1337-1341.

Ouma, J., El-Khoby, T., Fenwick, A., Blanton, R. (2001). Disease in schstosomiasis mansoni in Africa. In: Mahmoud, A. A. F. (Ed), Schistosomiasis. Imperial College Press, Lodon, pp. 333-360.

Pereira, L. M., Melo, M. C., Lacerda, C., Spinelli, V., Domínguez, A. L., Massarolo, P., Miles, S., Saleh, M. G., McFarlane, I. G., Williams R. (1994). *J. Med. Virol*, **42** (2): 203-206.

Pritchard, D. I., Quinnell, R. J., McKean, P. G. (1991). Antigenic cross-reactivity between N. americanus and *Ascaris lumbricoides* in a community in Papua New Guinea infected predominantly with hookworm. *Trans Roy Soc Trop Med Hyg,* **85**: 511–514.

Quinnell, R. J., Pritchard, D. I., Raiko, A., Brown, A. P., Shaw, M. A. (2004). Immune responses in human necatoriasis: association between interleukin-5 responses and resistance to reinfection. *J Infect Dis,* **190**: 430–438.

Richter, J., Zwingenberger, K., Ali, Q. M., Lima, W. M., Dacal, A. R., de Siqueira, G. V., Doehring-Schwerdtfeger, E., Feldmeier, H. (1992). Hepatosplenic schistosomiasis: comparison of sonographic findings in Brazilian and Sudanese patients--correlation of sonographic findings with clinical symptoms. *Radiology,* **184** (3): 711-716.

Richter, J., Hatz, C., Campagne, G., Berquist, N., Jenkins, J. (2000). Ultrasound in schistosomiasis: A Practical Guide to the Standardized use of Ultrasonography for the Assessment of Schistosomiasis Related Morbidity. World Health Organization. WHO/TDR/STR/SCH/001, Geneva. www.who.int/tdr.

Sacko, M. (2006). Assessment of the effect of two different treatment strategies on morbidity caused by *Schistosoma haematobium* infections among school children in the Niger river basin, Mali. PhD thesis, Faculty of Science, University of Copenhagen, Denmark.

Secor, W. E., del Corral, H., dos Reis, M. G., Ramos, E. A., Zimon, A. E., Matos, E. P., Reis, E. A., do Carmo, T. M., Hirayama, K., David, R. A., David, J. R., Harn, D. A. (1996). Association of hepatosplenic schistosomiasis with HLA-DQB1*0201. *J. Infect. Dis,* **174** (5): 1131-1135.

Stothard, J. R., Gabrielli, A. F. (2007). Schistosomiasis in African infants and preschool children: to treat or not to treat? *Trends Parasitol,* **23** (3): 83-86.

Sowunmi, A. (1996). Hepatomegally in acute faciparum malaria in children. *Trans. Roy. Soc. Trop. Med. Hyg,* **90**, 540-542.

Thapar, N., Sanderson, I. R. (2004). Diarrhoea in children: An interface between developing and developed countries. *Lancet*, **363:** 641-653.

Timothy, L. M., Coulson, P. S., Behnke, J. M., Wilson, R. A. (1992). Cross-reactivity between N. americanus and *Schistosoma mansoni* in mice. *Int J Parasitol,* **22**: 1143 – 1149.

Tomayo, S. G., Rickman, L. S., Mathews, W. C., Fullerton, S. C., Bartok, A, E., Wamer, J. T., Feigal, D. W., Amstain, D. G., Callander, N. S., Lyche, K. D (1993). Examinor dependence of physical diagnodtic tests of splenomegaly: A prospective study with multiple observers. *J. Gen. Int. Med*, **8** (2): 69-75.

Traore, M., Traore, H. A., Kardorff, R., Diarra, A., Landoure, A., Vester, U., Doehring, E., Bradley, D. J. (1998). The public health significance of urinary schistosomiasis as a cause of morbidity in two districts in Mali. *Am. J. Trop. Med. Hyg,* **59** (3): 407-413.

Tshikuka, J. G., Scott, M. E., Gray-Donald, K., Kalumba, O. N. (1996). Multiple infection with plasmodium and helminths in communities of low and relatively high socio-economic status. *Ann Trop Med Parasitol*, **90:** 277-293.

Ukoli, F. M. A. (1984). Schistosoma and Schistosomiasis. In: Introduction to parasitology in tropical Africa. (Ed.). John Willey&Sons Ltd. Norwich: UK. pp 52-89.

Utzinger, J., Bergquist, R., Shu-Hua, X., Singer, B. H., Tanner, M. (2003). Sustainable schistosomiasis control-the way forward. *The lancet*, **362:** 1932-1934.

Vennervald, J. B., Ouma, J. H., Butterworth, A. E. (1998). Morbidity in schistosomiasis: Assessmant, mechanisms and control. *Parasitol today*, Vol. 14, No. **10**, 1998.

Vennervald, J. B., Kenty, L., Butterworth, A. E., Kariuki, C. H., Kadzo, H., Ireri, E., Amaganga, C., Kimani, G., Mwatha, J., Otedo, A., Booth, M., Ouma, J. H., Dunne, D. W. (2004a). Detailed clinical and ultrasound examination of children and adolescents in *Schistosoma mansoni* endemic areas in Kenya: Hepatosplenic disease in the absence of portal fibrosis. *Trop Med Int Health*, **9** (4): 1-10.

Vennervald, B. J and David W. Dunne (2004b). Morbidity in schistosomiasis. *Cur Opin Infect Dis*, **17**: 439-447.

Warrel, D., Turner, G. D. H., Francis, N. (2002). Pathology and pathophysiology of human malaria. In: Essenetial malariology. 4th Edition, 2000 Arnold. London: UK. pp 236-251.

Warren, K. (1987). Schistosomiasis: Past, Present and Future. Mem. Inst. Oswaldo Cruz, 82, suppl. IV, 15-29.

Whittle, H., Gelfand, M., Sampson, E., Purvis, A., Weber, M. (1969). Enlarged livers and spleens in an area endemic for malaria and schistosomiasis. *Trans. Roy. Soc. Trop. Med. Hyg*, **63** (3): 353-361.

Wilson, S., Vennervald, B. J., Kadzo, H., Ireri, E., Amaganga, C., Booth, M., Kariuki, H. C., Mwatha, J. K., Kimani, G., Ouma, J., Muchiri, E., Dunne, D. (2007). Hepatosplenomegaly in Kenyan school children: Exacerbation by concurrent chronic exposure to malaria and *schistosma mansoni* infection. *Trop. Med. Int. Health*, **12**: 1442-1449.

Yazdanpanah, Y., Thomas, A. K., Kardorff, R., Talla, I., Sow, S., Niang, M., Stelma, F. F., Decam, C., Rogerie, F., Gryseels, B., Capron, A., Doehring, E. (1997). Organometric investigations of spleen and liver by ultrasound in *schistosoma mansoni* endemic and non-endemic villages in Senegal. *Am. J. Trop. Med. Hyg*, **57** (2):245-249.

Chapter 5: The impact of two anthelmintic treatment approaches on malaria infection, anaemia and on schistosome and STH infections among school and preschool children in Magu district, Tanzania

Abstract

A study was undertaken to assess the impact of two anthelmintic treatment approaches on malaria infection, anaemia and on schistosome and STH infections among school and preschool children in Magu district, Tanzania. A total of 765 helminth infected children were selected from 1546 children, who were examined at baseline, and included into a prospective anthelmintic intervention study. The 765 children were randomized to either receive treatment with praziquantel (PZQ) and albendazole (ALB) four times a year (intervention group 394 children) or to receive treatment with the standard single dose of PZQ and ALB once a year (control group 371 children). Two follow up surveys were conducted at 12 and 24 months after baseline to assess the impact of the intervention. Stool and urine samples were collected and examined for schistosome and STH eggs. Blood samples were collected and examined for malaria parasitaemia and Hb levels. Ultrasound examination of the abdomen and urinary tract was performed on all children to assess for *S. mansoni* and *S. haematobium* related pathology, respectively. In addition, monitoring of clinical malaria attacks was performed at each school during the two years of the intervention. Baseline survey showed that 1079 (69.8%) children were infected with at least one of the parasites *P. falciparum, S. mansoni, S. haematobium,* hookworm and *T. trichiura,* out of whom 430 (39.9%) harbored more than one parasite species. Overall, the intervention did not have an impact on malaria infection (prevalence, malaria parasite density and frequency of clinical malaria attacks) or on prevalence of anaemia compared to the control. However, the intervention significantly reduced prevalence and intensity of *S. mansoni, S. haematobium* and hookworms compared to the standard single dose annual treatment ($p < 0.001$). Overall, there was 100% reduction in the prevalence of *S. haematobium* related urinary bladder pathology for the intervention and control groups over the two years follow up period. Further, there was significant reductions in the prevalence of multiple parasite infections over the two years follow up period with significant differences between groups ($p < 0.001$). There were reductions in the prevalence of periportal fibrosis, splenomegaly, hepatosplenomegaly and enlarged portal vein diameter over the two years follow up period but without significant differences between groups. There was no significant reductions in the prevalence of enlarged left liver lobe (hepatomegaly) over the two years follow up period ($\chi^2 = 0.035$, $p = 0.851$). Overall there was significant improvement in mean Hb concentrations from baseline level of 124 (95% CI 123-125) to 136 (95% CI 134-138) and 137 (95% CI 135-139) in the intervention and control groups, respectively at 2nd annual follow up ($t = -18.10$, $p < 0.001$) which in turn resulted into a significant reduction of the prevalence of anaemia. These results indicate that an antihelminic intervention to control helminth infections may not have a direct impact on malaria parasitaemia, infection density or clinical malaria. However the intervention have a sinigificant impact on overall prevalence of single and multiple infections.

5.1. Introduction

Malaria, schistosomiasis and soil transmitted helminth infections (STH) are considered the most important parasitic infections in Sub-Saharan Africa, contributing to the biggest share of clinical disease burden (Chitsulo *et al*, 2000; WHO, 2002). The diseases share the same geographical distribution and occur as co-infections in humans and thus interact with regards to susceptibility, infection level, and pathology (De Silva *et al*, 2003; Utzinger *et al*, 2004). In school children, the diseases are associated with impaired physical and mental development, impaired learning capabilities, severe undernutrition and anaemia (Stephenson *et al*, 2000, WHO, 2002; Hotez, 2004). Previous studies have demonstrated that helminth infections increase the risk of infections with *P. falciparum* (Tshikuka *et al*, 1996; Spiegel *et al*, 2003; Sokhna *et al*, 2004; Le Hesran *et al*, 2004; Maizels *et al*, 2004) and other infections such as HIV and TB (Gallagher *et al*, 2005). Another important aspect of malaria and helminth co-infections in humans is their joint contribution to anaemia (Dreyfus *et al*, 2000). A study in Nepal (Dreyfuss *et al*, 2000) observed that *P. vivax* malaria and hookworm co-infections in pregnant women were associated with more frequent malaria attacks and severe anaemia than seen in women who harbour only one parasite infection. In the Philippines it was demonstrated that even at low infection intensities, multiple parasite infections enhance the risk of anaemia (Ezeamama *et al*, 2005; Ezeamama, 2008). Thus an intervention to control helminth infections in areas where helminth infections are co-endemic with other infections could be expected to have not only an impact on prevalence and intensity of helminth infections but also an impact on disease burden due to malaria and other infections (Tohon *et al*, 2008). However, there has been no longitudinal community based randomized studies to test these hypotheses. Most studies have been cross sectional in nature. Thus the major objective of this study was to assess the impact of an anthelmintic intervention delivered through two different approaches on malaria infection, anaemia and on the prevalence and infection intensity of schistosomiasis, STH and on schistosomiasis related morbidity.

5.2. Methodology

5.2.1. Study population and design

A total of 765 children were selected from the 1546 children examined at baseline and included in a longitudinal anthelmintic intervention study. Children selected were those infected with either *S. mansoni or S. haematobium* or both. Selected children were randomized into either the intervention group (394 children) or control group (371 children). The intervention group was treated with praziquantel 40mg/kg and albendazole 400mg four times a year at three months interval while the control group was treated with praziquantel 40mg/kg and albendazole 400mg once a year. Two follow up surveys were conducted. The first follow up survey (12 months after baseline) was conducted between October and November 2007 and involved 655 children. The second follow up survey (24 months after baseline) was conducted between October and November 2008 and involved 592 childreen. More details about selection of children, randomization, treatments, baseline and follow up surveys are given in Chapter 2.

5.2.2. Data analysis

Data collected were analyzed using STATA Version 10. Infection intensities (of positive samples only) were calculated as geometric mean of eggs per gram of faeces for *S. mansoni* and hookworm infections, eggs per 10ml of urine for *S. haematobium* and parasites per microlitre of blood for *P. falciparum*. The student's t-test and one way analysis of variance (ANOVA) was used to compare geometric mean parasite counts (in parasite positive samples only) and mean haemoglobin concentrations (in all samples examined) between groups where two or more than two groups were compared, respectively. Geometric mean parasite counts (positive samples only) and their corresponding 95% confidence intervals (95% CI) were calculated using STATA version 10. The Chi-square test was used to compare proportions (prevalence) of schistosomiasis, STH, malaria and anaemia between groups. Graphs were drawn using STATA 10 or MS-Excel as appropriate. Tests were considered statistically significant at $p < 0.05$.

5.3. Results

5.3.1. Baseline characteristics

Out of the 765 children included in the longitudinal study, 655 children (85.6%) were successfully traced and examined during the first annual follow up survey conducted between October and November 2007. Out of these, complete information was available for 646 children of whom 319 (49.4%) were boys. Mean age was 8 years. During the second annual follow up survey conducted between October and November 2008, a total 592 or 77.4% of all children included in the longitudinal follow up were traced and examined. Complete information was available for 589 children out of whom 291 (49.4%) were boys. Mean age was 9 years. Table 5.1 shows baseline characteristics of children included in the longitudinal study.

Table 5.1 Baseline characteristics of children included in the longitudinal study by randomization group (n = 765).

Parameter/category	Intervention group (n = 394)	Control group (n = 371	P - Value
Sex			
Boys (n = 392)	193 (49.0%)	180 (48.5%)	
Girls (n = 373)	201 (51.0%)	191 (49.0%)	0.897
Age distribution (years)			
3 – 5 (n = 93)	40 (10.1%)	53 (14.3%)	
6 – 8 (n = 517)	278 (70.6%)	239 (64.4%)	
9 – 13 (n = 155)	76 (19.3%)	79 (21.3%)	0.127
S. mansoni infection			
Prevalence (%)	238 (60.4)	220 (59.3)	0.755
Intensity (Epg)*	49 (42-59)	50 (42-59)	0.706
S. haematobium infection			
Prevalence (%)	90 (22.8)	85 (22.9)	0.974
Intensity (Eggs/10ml)*	15 (11-20)	17 (13-23)	0.335
S. mansoni/S. haematobium			
Prevalence (%)	65 (16.6)	61 (16.4)	0.915
S. mansoni intensity (Epg)*	31 (23-42)	41 (29-58)	0.762
S. haemat intensity (egg/10ml)*	19 (11-28)	16 (10-26)	0.221
Malaria infection			
Prevalence (%)	123 (31.2)	123 (33.2)	0.567
Parasite density (mps/µL)*	530 (414-676)	510 (403-644)	0.825
Hookworm infection			
Prevalence (%)	80 (20.3)	68 (18.3)	0.489
Intensity (Epg)*	54 (39-76)	53 (39-73)	0.923
Anaemia			
Prevalence (%)	147 (37.3)	138 (37.2)	0.971
Haemoglobin level (g/L)**	122 (121-123)	123 (122-124)	0.270
Multiple infections (%)			
Single parasite	199 (50.5)	186 (50.1)	
Two parasites	148 (37.6)	136 (36.7)	
Three/four parasites	47 (12.0)	49 (13.2)	0.862

***Infection intensity expressed as geometric mean parasite counts of positive samples only with 95% confidence interval shown in brackets.**

****Mean haemoglobin concentrations with 95% confidence interval shown in brackets.**

Out of the 646 children who had complete information at first annual follow up survey, 433 (67%) were infected with at least one of the parasites *P. falciparum, S. mansoni, S. haematobium,* hookworm and *T. trichiura.* One hundred and fifty four children (35.6%) were infected with more than one parasites. Out of the 282 who were infected with *P. falciparum*, 129 (45.7%) had helminth co-infections.
At the 2nd annual follow up survey, 589 children with complete information were included in analysis and out of these 302 (51.4%) were infected with at least one of the parasites *P. falciparum, S. mansoni, S. haematobium,* Hookworm and *T. trichiura*. Children who were infected with *P. falciparum* were 144 (24.5%), out of whom 55 (38.2%) had helminth co-infections.

5.3.2. Impact of the two treatment regimens on malaria infection, Hb levels and anaemia after one and two years of antihelmintic treatment

There was no significant difference in malaria infection (prevalence and malaria parasite density) and in the prevalence of anaemia between the intervention and control groups ($p > 0.05$) at the first and second follow up surveys. However, there was overall significant improvement in mean haemoglobin concentrations ($t = -18.10$, $p < 0.001$) from baseline level of 124 (95% CI 123-125) to 136 (95% CI 134-138) and 137 (95% CI 135-139) in the intervention and control groups, respectively during the 2nd annual follow up survey which in turn resulted into a significant reduction in the prevalence of anaemia over the two years follow up period but without significant differences between groups (Table 5.2).

Table 5.2 Comparison of overall prevalence and geometric mean parasite density of malaria infection, mean Hb levels and anemia after one and two years of antihelmintic treatment by randomization groups

Survey period/ Parameter	Prevalence (%)			Geometric mean malaria parasite density/Hb levels (95% CI)		
	Intervention	**Control**	**P- Value**	**Intervention**	**Control**	**P-Value**
Baseline (n = 765)						
P. falciparum infection	123 (33.2)	123 (31.2)	0.567	530 (414-678)	510 (403-645)	0.825
Anaemia	142 (36.0)	134 (36.1)	0.971	110 (108-111)	112 (110-113)	0.044
Severe anaemia	5 (1.3)	4 (1.1)		70 (49-94)	63 (46-84)	0.497
1st Follow up (n = 646)						
P. falciparum infection	145 (44.9)	137 (42.4)	0.526	833 (664-1048)	906 (724-1133)	0.608
Anaemia	120 (37.2)	112 (34.7)	0.806	111 (109-112)	110 (108-112)	0.695
Severe anaemia	1 (0.3)	1 (0.3)		NA	NA	
2ndFollow up (n = 589)						
P. falciparum infection	74 (24.9)	70 (24.0)	0.790	326 (279-382)	343 (294-400)	0.658
Anaemia	43 (14.5)	38 (13.0)	0.634	111 (109-112)	112 (110-114)	0.386
Severe anaemia	0	1 (0.3)		NA	NA	

NA = not applicable

There was a significant reduction in the prevalence of light and heavy *P. falciparum* infections over the two years follow up period ($\chi^2 = 5.92$, $p = 0.015$) but without significant differences between groups ($\chi^2 = 0.07$, $p = 0.790$). Likewise, there was an overall significant reduction in malaria parasite density ($t = -5.37$, $p < 0.001$) over the two years follow up period but without significant differences between groups ($t = 0.44$, $p = 0.658$).

5.3.3. Impact of the antihelmintic treatment intervention on clinical malaria atttacks

A total of 162 children presented with symptoms suggestive of malaria (axillary temperature $\geq$ 37.5^0C) over the two years follow up period. However only 91 children (56.2%) were confirmed malaria cases (axillary temp $\geq$ 37.5% plus a bood smear positive for malaria parasites). Fourty nine malaria cases (55.1%) were reported from the intervention group while 42 malaria cases (57.5%) were reported from the control group. On average, a child had 2 malaria attacks (range 1 - 4) per year for the first follow up year (2007) and 1 malaria attack (range 1 - 2) per year in the second year of follow up (2008).

Table 5.3 Distribution of clinical malaria attacks and geometric mean malaria parasite density by treatment and age group over 2 years (n = 162).

Variable/age group	**Number of malaria attacks (%)**		**P-Value**
	Intervention group (n = 89)	**Control group (n = 73)**	
Malaria attacks			
5 – 8 yrs (n = 105)	38 (61.3)	24 (55.8)	0.575
9 – 15 yrs (n = 57)	11 (40.7)	18 (60.0)	0.146
Total (n = 162)	49 (55.1)	42 (57.5)	0.752
Malaria parasite density*			
5 – 8 yrs (n = 105)	4170 (2400-7240)	5397 (2797-9414)	0.934
9 – 15 yrs (n = 57)	1102 (470-2590)	1480 (652-3360)	0.892
Total (n = 162)	2658 (1647-4291)	3100 (1831-5249)	0.451

***Malaria parasite density expressed as geometric mean malaria parasites per microlitre of blood (positive samples only) with 95% confidence intervals shown in brackets.**

Overall, there was no significant difference in frequency of malaria attacks ($\chi^2 = 0.10$, p = 0.752) or malaria parasite density (t = -0.76, p = 451) between children in the intervantion group and those in the control group (table 5.3).

5.3.4. Impact of the two treatment regimens on prevalence and intensity of schistosome and hookworm infections at first and second follow-up.

Table 5.4 Comparison of prevalence and infection intensity (expressed as geometric mean parasite count of positive samples only) of schistosome and hookworm infections at first and second follow-up by randomization groups

Survey period	Prevalence (%)			Infection intensity (Geometric mean parasite count (95% CI))		
	Intervention	**Control**	**P-Value**	**Intervention**	**Control**	**P-Value**
Baseline (n = 765)						
S. mansoni	303 (76.9)	281 (75.7)	0.705	50 (42-59)	50 (42-60)	0.941
S. haematobium	155 (39.3)	146 (39.4)	0.997	15 (11-21)	17 (13-23)	0.574
Hookworm	80 (20.3)	68 (18.3)	0.489	53 (39-76)	54 (40-73)	0.923
1st Follow up (n = 646)						
S. mansoni	85 (26.3)	143 (44.3)	< 0.001	32 (24-44)	40 (33-50)	0.227
S. haematobium	15 (4.7)	42 (13.0)	< 0.001	2 (1-3)	11 (6-20)	< 0.001
Hookworm	8 (2.5)	33 (10.2)	< 0.001	25 (7-92)	35 (22-58)	0.538
2ndFollow up (n = 589)						
S. mansoni	65 (21.9)	124 (42.5)	< 0.001	35 (24-52)	36 (27-46)	0.975
S. haematobium	4 (1.4)	17 (5.8)	0.003	6 (1-24)	9 (4-20)	0.418
Hookworm	3 (1.0)	13 (4.5)	0.010	55 (1-634)	40(19-84)	0.707

Table 5.4 shows that overall, there was significant reduction in prevalence of *S. mansoni*, *S. haematobium* and hookworms infections between the intervention and control group over the two years follow up period. Infection intensities tended to be lower in the intervention group compared to the control group without being statistically significant except for *S. haematobium* infection ($t = 3.72$, $p<0.001$) at 1st follow up examination.

Table 5.5 Comparison of prevalence and infection intensity (expressed as geometric mean parasite count of positive samples only) of *S. mansoni*, *S. haematobium* and hookworms by sex and randomization group and first and second follow-up.

Survey period/ Infection	Sex	Prevalence (%)			Infection intensity (Geometric mean parasite count (95% CI)		
		Intervention	Control	P-Value	Intervention	Control	P-Value
Baseline (n = 765)							
S. mansoni	Boys	150 (77.7)	133 (73.9)	0.387	55 (43-70)	56 (43-74)	0.923
	Girls	153 (76.1)	148 (77.5)	0.749	45 (35-57)	45 (37-56)	0.954
	P-Value	0.706	0.419		0.953	0.923	
S. haematobium							
	Boys	76 (39.4)	79 (43.9)	0.377	15 (10-22)	23 (15-33)	0.099
	Girls	79 (39.3)	67 (35.1)	0.387	16 (10-25)	13 (8-19)	0.325
	P-Value	0.988	0.083		0.632	0.029	
Hookworm							
	Boys	41 (21.2)	33 (18.2)	0.481	77 (48-122)	66 (44-98)	0.593
	Girls	39 (19.4)	35 (18.3)	0.785	38 (24-61)	44 (28-71)	0.665
	P-Value	0.650	0.998		0.035	0.199	
1st Follow up (n = 646)							
S. mansoni	Boys	37 (22.8)	63 (40.1)	< 0.001	25 (16-38)	40 (29-56)	0.075
	Girls	48 (29.8)	80 (48.2)	< 0.001	40 (26-61)	40 (31-54)	0.934
	P-Value	0.155	0.145		0.139	0.990	
S. haematobium							
	Boys	6 (3.7)	26 (16.6)	< 0.001	2 (1-4)	11 (6-23)	0.014
	Girls	9 (5.6)	16 (9.6)	< 0.014	2 (1-4)	11 (3-34)	0.020
	P-Value	0.421	0.065		0.644	0.948	
Hookworms							
	Boys	5 (3.1)	18 (11.5)	< 0.001	15 (6-42)	32 (17-60)	0.236
	Girls	3 (1.8)	15 (9.0)	< 0.001	57 (16-213)	40 (17-96)	0.753
	P-Value	0.479	0.471		0.279	0.220	
2nd Follow up (n = 589)							
S. mansoni	Boys	33 (22.1)	62 (43.7)	< 0.001	41 (23-73)	44 (29-65)	0.872
	Girls	32 (21.6)	62 (41.3)	< 0.001	30 (17-53)	29 (20-42)	0.914
	P-Value	0.913	0.687		0.424	0.129	
S. haematobium	Boys	1 (0.7)	7 (4.9)	< 0.001	13	14 (4-45)	NA-
	Girls	3 (2.0)	10 (6.7)	< 0.001	4 (1-43)	7 (2-24)	0.502
	P-Value	0.311	0.526		0.179	0.440	
Hookworm	Boys	2 (1.3)	7 (4.9)	< 0.001	31 (3-387)	40 (10-160)	0.883
	Girls	1 (0.7)	6 (4.0)	< 0.001	168	169	-NA
	P-Value	0.566	0.700		0.277	0.220	

NA = not applicable

Table 5.5 Shows that there was significant reduction in prevalence of *S. mansoni*, *S. haematobium* and hookworm infections for the intervention group compared to the control group over the two years follow up period but without significant differences between boys and girls in each group. Infection intensities tended to be lower for the intervention group compared to the control group but the differences were not statistically significant except for *S. haematobium*.

Table 5.6 Comparison of changes in prevalence and infection intensity (expressed as geometric mean parasite count of positive samples only) of *S. mansoni, S. haematobium* and hookworm by age groups at first and second follow-up.

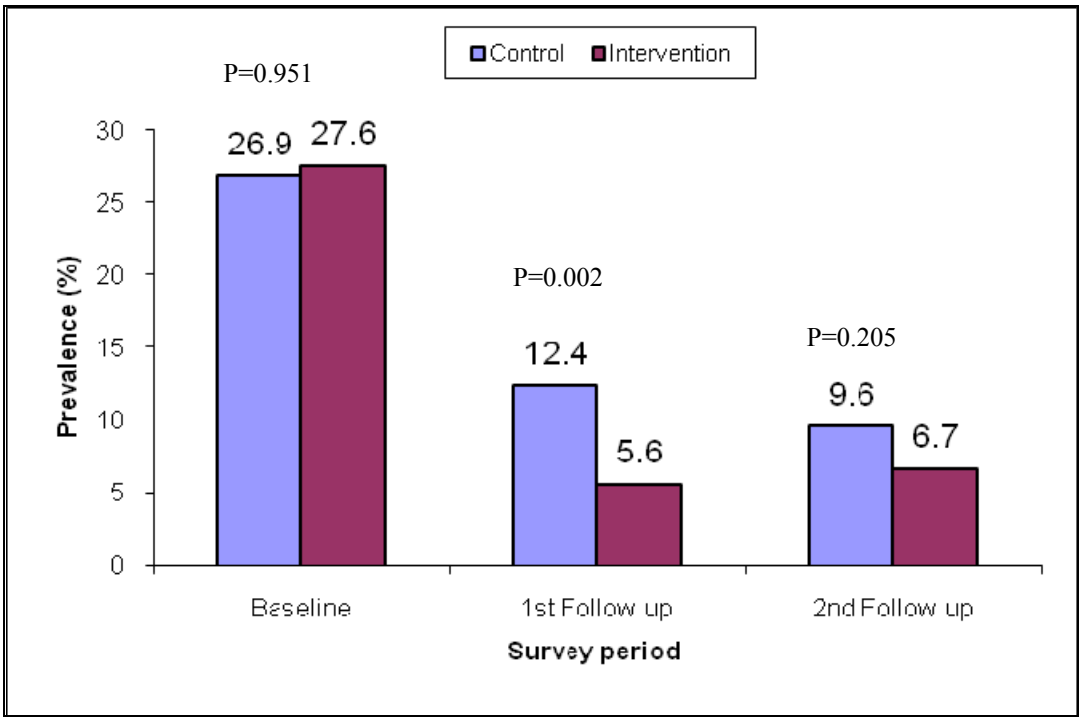
Survey period/ Infection	Age group	Prevalence (%)			Infection intensity (Geometric mean parasite count (95% CI))		
		Intervention	Control	P-Value	Intervention	Control	P-Value
Baseline	3 – 5	23 (57.5)	36 (67.9)	0.301	51 (27-93)	42 (26-70)	0.642
(n = 765)	6 – 8	221 (79.5)	180 (75.3)	0.256	47 (38-58)	45 (37-56)	0.763
S. mansoni	9 - 13	59 (77.6)	65 (82.3)	0.470	59 (40-87)	76 (52-109)	0.376
	P-Value	0.008	0.163		0.584	0.039	
S. haematobium	3 – 5	20 (50.0)	22 (41.5)	0.451	19 (7-47)	26 (11-60)	0.587
	6 – 8	101 (36.3)	93 (38.9)	0.546	15 (11-22)	18 (13-25)	0.607
	9 - 13	34 (44.7)	31 (39.2)	0.488	13 (9-25)	11 (5-23)	0.742
	P-Value	0.143	0.940		0.763	0.204	
Hookworm	3 – 5	8 (20.0)	7 (13.2)	0.318	23 (6-92)	43 (14-128)	0.425
	6 – 8	51 (18.4)	41 (17.2)	0.462	61 (40-93)	47 (32-68)	0.341
	9 - 13	21 (27.6)	20 (25.3)	0.713	56 (29-110)	76 (40-146)	0.506
	P-Value	0.204	0.155		0.249	0.320	
1st Follow up							
(n = 765)	4 – 6	7 (18.9)	18 (40.9)	< 0.001	40 (5-310)	82 (39-176)	0.371
S. mansoni	7 – 9	62 (27.8)	93 (44.3)	< 0.001	33 (39-176)	35 (27-44)	0.831
	10 - 14	16 (25.4)	32 (46.4)	< 0.001	27 (14-51)	42 (26-67)	0.252
	P-Value	0.515	0.850		0.783	0.026	
S. haematobium-	4 – 6	1 (2.7)	7 (15.9)	< 0.001	4	25 (8-92)	NA-
	7 – 9	11 (4.9)	29 (13.8)	< 0.001	2 (1-3)	11 (6-24)	0.002
	10 - 14	3 (4.7)	6 (8.7)	0.080	2 (1-17)	4 (1-34)	0.586
	P-Value	0.836	0.450		0.628	0.275	
Hookworms	4 – 6	1 (2.7)	3 (6.8)	0.109	865	90 (10-858)	NA-
	7 – 9	6 (2.7)	16 (7.6)	< 0.001	16 (7-34)	33 (19-57)	0.128
	10 - 14	1 (1.6)	14 (20.9)	< 0.001	12	32 (12-89)	NA-
	P-Value	0.880	0.008		0.283	0.499	
2nd Follow up	5 – 7	4 (11.4)	16 (40.0)	< 0.001	45 (4-484)	37 (16-82)	0.811
(n = 589)	8 – 10	48 (23.8)	85 (44.3)	< 0.001	37 (22-61)	31 (22-43)	0.536
	11 - 15	13 (21.7)	23 (38.3)	< 0.001	26 (13-53)	57 (33-98)	0.087
S. mansoni	P-Value	0.265	0.679		0.797	0.260	
S. haematobium	5 – 7	1 (2.8)	2 (5.0)	0.320	2	1	NA-
	8 – 10	2 (1.0)	11 (7.7)	< 0.001	11 (2-62)	16 (7-34)	0.555
	11 - 15	1 (1.7)	4 (6.8)	< 0.001	5	7 (1-75)	NA-
	P-Value	0.657	0.937		0.259	0.051	
Hookworm	5 – 7	0	2 (5.0)	NA-	0	68 (1-554)	NA-
	8 – 10	1 (0.5)	5 (2.6)	< 0.01	6	38 (5-285)	NA-
	11 – 15	2 (3.3)	6 (10.0)	< 0.01	165 (130-208)	36 (11-115)	0.115
	P-Value	0.143	0.047		0.006	0.834	

For all age groups, there was a significant reduction in prevalence of *S. mansoni*, *S. haematobium* and hookworm infections for the intervention group compared to the control group over the two

years follow up period. Infection intensity tended to be lower for the intervention group as compared to the control group but without statistically significant differences.

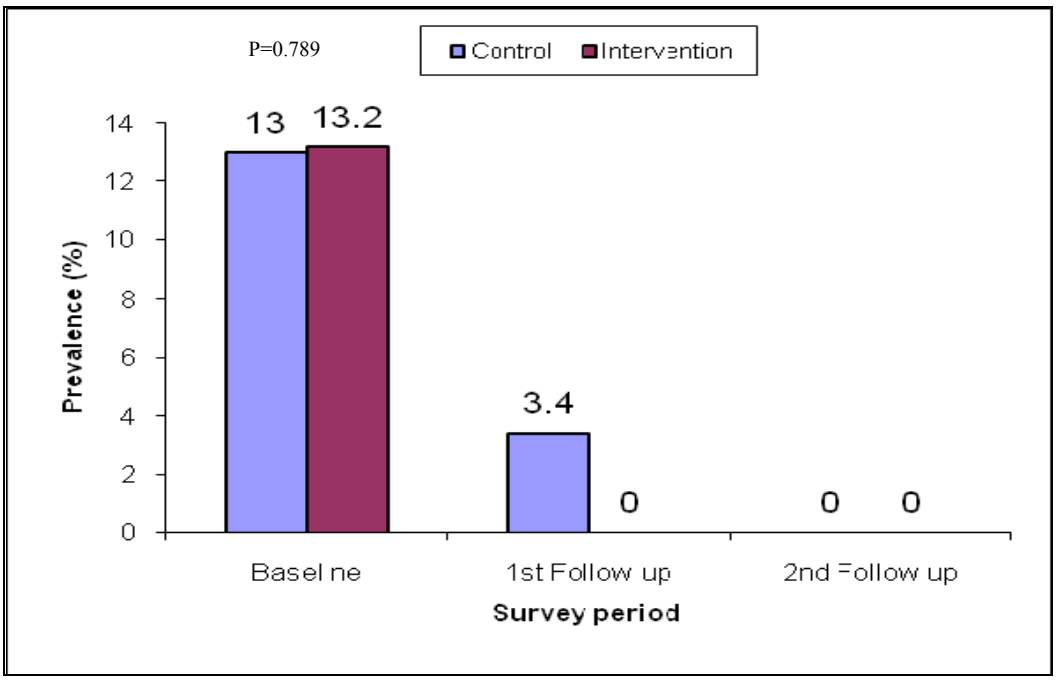
5.3.5. Impact of the two treatment regimens on prevalence of light and heavy *S. mansoni, S. haematobium* infections one year and two years after antihelmintic treatment

Figure 5.1 Comparison of prevalence of moderate to heavy *S. mansoni* infection by randomization group at 1st annual follow up survey (n = 646) and 2nd annual follow up survey (n = 589).



There was a significant reduction (χ^2 = 9.17, p = 0.002) in prevalence of moderate to heavy *S. mansoni* infection for the intervention group during the first annual follow up but not the second annual follow up (Figure 5.1).

Figure 5.2 Comparison of prevalence of heavy *S. haematobium* infection by randomization group at 1st follow up survey (n = 646) and second follow up survey (n = 589).

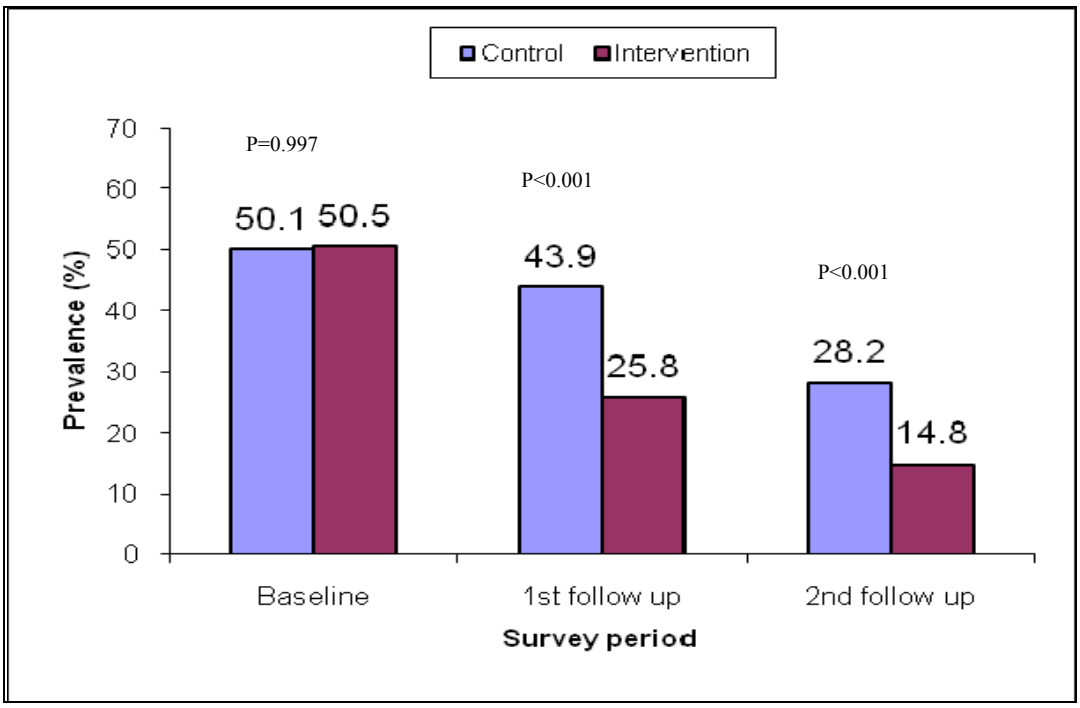


There was 100% reduction in prevalence of heavy *S. haematobium* infection for the intervention group during the first annual follow up and 100% reduction for all groups during the second annual follow up (Figure 5.2).

5.3.6. Impact of the two treatment regimens on prevalence of multiple parasite infections at first and second follow-up.

At baseline, 430 or 39.9% of all infected children harbored two or more parasite species. Figure 5.3 shows the prevalence of multiple (two or more) parasite infections during the first and second annual follow up surveys in relation to baseline levels.

Figure 5.3 Prevalence of multiple parasitic infections by randomization group at 1st follow up (n = 646) and at 2nd follow up (589).



Overall, there was significant reductions in prevalence of multiple parasite infections during the first and second annual follow up (Figure 5.3).

5.3.7. Impact of the two treatment regimens on prevalence of different morbidity indicators at first and second annual follow-up

At baseline 1258 children (81.4%) had at least one of the *S. mansoni* related pathology while at 67 children (4.3%) had at least one of the *S. haematobium* related pathology. Table 9 shows a comparison of different morbidity indicators by randomization group over the two years of the antihelmintic intervention.

Table 5.7 Prevalence (%) of different *S. mansoni* related morbidity indicators by randomization group at one and two years after antihelmintic intervention (n = 1546).

Parameter	Baseline (n = 765)	1st Follow up (n = 646)	2nd Follow up (n = 589)
Periportal fibrosis (PPF)			
Intervention	11 (2.8)	5 (1.6)	2 (0.67)
Control	8 (2.2)	6 (1.9)	0
P-Value	0.562	0.716	NA
Hepatomegaly			
Intervention	297 (75.4)	279 (86.4)	230 (77.4)
Control	307 (83.0)	288 (89.2)	233 (79.8)
P-Value	0.108	0.280	0.486
Splenomegaly			
Intervention	164 (41.6)	143 (44.2)	86 (29.0)
Control	155 (41.8)	146 (45.2)	79 (27.0)
P-Value	0.968	0.812	0.607
Hepatosplenomegaly			
Intervention	130 (33.0)	131 (40.6)	76 (25.6)
Control	130 (35.0)	133 (41.2)	74 (25.3)
P-Value	0.551	0.873	0.945
Enlarged PVD			
Intervention	59 (14.9)	26 (8.1)	5 (1.7)
Control	58 (15.6)	28 (8.7)	6 (2.2)
P-Value	0.832	0.776	0.739

NA means not applicable

Overall, there was significant reduction of the prevalence of periportal fibrosis, splenomegaly, hepatosplenomegaly and of enlarged portal vein diameters over the two year follow up period without significant differences between groups. However, the overall prevalence of hepatomegaly remained fairly the same. There was complete (100%) resolution of *S. haematobium* related urinary bladder pathology for the intervention and control groups over the two follow up years.

Table 5.8 Size of height adjusted left liver lobe (Liver PSL in cm), spleen (spleen lengh in cm) and portal vein diameter (PVD in mm) (expressed as mean and 95% confidence intervals in brackets) by randomization group at one and two years after antihelmintic intervention.

Parameter	**Baseline (n = 765)**		**1st Follow up (n = 646)**		**2nd Follow up (n = 589)**	
	101-120cm	121-140cm	101-120cm	121-140cm	101-120cm	121-140cm
Liver PSL						
Intervention	7.6 (7.4-7.7)	8.0 (7.8-8.2)	8.1 (7.8-8.3)	8.3 (8.1-8.4)	7.6 (7.1-8.1)	8.1 (8.0-8.3)
Control	7.6 (7.4-7.8)	8.2 (7.9-8.3)	7.8 (7.6-8.0)	8.4 (8.1-8.5)	7.8 (7.3-8.3)	8.3 (8.1-8.4)
P-Value	0.783	0.123	0.164	0.304	0.604	0.111
Spleen length						
Intervention	8.4 (8.2-8.7)	9.3 (9.1-9.6)	8.6 (8.2-9.1)	9.2 (9.0-9.4)	7.8 (7.2-8.4)	8.7 (8.5-8.9)
Control	8.4 (8.2-8.6)	9.3 (9.0-9.6)	8.4 (8.1-8.8)	9.2 (9.0-9.5)	7.9 (7.3-8.6)	8.7 (8.5-8.9)
P-Value	0.710	0.949	0.407	0.858	0.723	0.923
PVD						
Intervention	6.9 (6.7-7.0)	7.3 (7.2-7.5)	6.8 (6.5-7.0)	7.2 (7.1-7.3)	6.7 (6.3-7.0)	6.9 (6.8-7.0)
Control	6.9 (6.8-7.0)	7.4 (7.2-7.5)	6.9 (6.7-7.1)	7.2 (7.1-7.4)	6.6 (6.2-7.0)	7.0 (6.8-7.1)
P-Value	0.917	0.745	0.250	0.848	0.878	0.503

Table 5.8 shows that overall, for each height group, there were no significant reductions in the size of the spleen and portal vein diameter ($p < 0.05$) over the two years follow up period between intervention groups. However, the size of the left liver lobe remained unchanged.

5.4. Discussion

The impact of the interventions on malaria infection

Results of the current study suggest that the antihelmintic intervention did not have any impact on malaria infection (prevalence, malaria parasite density and frequency of malaria attacks) as no differences were observed between children in the anthelmintic intervention group compared to children in the control group. This is contrary to what was expected. Previous studies had observed that helminth infections increased the risk of malaria infection (Helmby *et al*, 1998; Spiegel *et al*, 2003; Nacher et *al*, 2002; Sokhna *et al*, 2004; Le Hesran *et al*, 2004) which could imply that an antihelmintic intervention would result into reduced frequency of malaria attacks and/or malaria parasite densities in areas where both malaria and helminth infections are endemic (Druilhe *et al*, 2005). One explanation of this finding could be that the impact of helminth infections on malaria infection is intensity dependent such that heavy helminth infections are required to induce a shift in Th1/Th2 balance which in turn would be reflected in differences in the observed malaria infection between children in the intervention and control groups. This view has also been supported by findings of the study of Sokhna *et al* (2004) in Senegal who observed that the incidence rate of malaria attacks was significantly higher in children infected with *S. mansoni* particularly those carrying the highest egg loads as compared to uninfected children. Another recent study by Waknine-Grinberg *et al* (2010) demonstrated that concomitant *S. mansoni* infection reduced the incidence of cerebral malaria in *P. bergheii* infected mice but the effect was dependent on *S. mansoni* parasite load. As most of the helminth infections in this study were light, their impact on malaraia infections might be weak. The results are however in agreement with findings of Beasley *et al* (1999) who worked with school children aged 7 – 12 years in Tanga Region in Northeast Tanzania where malaria, *S. haematobium* and STHs are co-endemic. He observed that 15 to 16 weeks following antihelmintic treatment using praziquantel and albendazole, children in the treatment group had significant reductions in prevalence and infection intensity of *S. haematobium* and STHs and in the prevalence of anaemia compared to children in the control (placebo) group but did not differ in terms of prevalence and intensity of infection of *P. falciparum* malaria.

One weakness of the design of the current study is the lack of an untreated control group due to ethical reasons. While children in the intervention group were treated with praziquantel 40mg/kg and albendazole 400mg four times a year, children in the comparison group had to be given the minimum recommended single dose of praziquentel 40mg/kg and albendazole 400mg annually according to the National Schistosomiasis and Soil Transmitted Helminths Control Programme. Thus the single dose annual anti-helminthic treatment of children in the control group might have contributed to lack of differences in malaria infection between children in the intervention group compared to the control group. An earlier study to investigate this relationship using a randomized design was that of Murray *et al* (1978) which suggested that treatment of children with helminth infections (*A. lumbricoides*) resulted into an increase of malaria cases. Two studies with similar design in Madagascar (Brutus *et al*, 2006; Brutus *et al*, 2007) showed that children aged more than 5 years treated for helminths (*Ascaris lumbricoides*) had a significant increase in malaria parasite densities compared to untreated controls suggesting a negative interaction between *A. lumbricoides* infection and malaria parasite density. It might be difficult to compare results of the current study with the studies of Murray and Brutus. While the study of Murray *et al* (1978) included a very small sample size (only 35 children were studied), all the three studies investigated the relationship between *P. falciparum* and *A. lumbricoides* infections which are different helminth species from the species investigated in the current study. The studies of Murray and Brutus were also conducted in a different epidemiological setting and used a different design in that the treatment against STH was either piperazine (Murray *et al*, 1978) or levamisole (Brutus *et al*, 2006, 2007) and no treatment was

given against schistosome infection. Overall, to date, studies on malaria and helminth co-infections both in animal models and in humans have produced conflicting results. Furthermore, most studies have been observational in nature, which make them prone to biases as they do not have sufficient mechanisms to control confounding variables such as location of residence, environmental factors as well as host and parasite genetics (Mwangi *et al*, 2006). The current study however was a randomised antihelmintic intervention study reducing the risk of bias and confounding.

Despite the lack of significant differences between the study groups, there was an overall reduction in prevalence of parasitaemia, intensity of infection and number of malaria attacks from baseline through to the second follow up survey. However, this reduction was not consistent over time since the prevalence and infection intensity of malaria infection was higher at the first follow up. The fact that this reduction was not consistent overtime suggests that other factors such as seasonal variation in malaria transmission resulting from malaria vector dynamics and climatic/environmental factors might have contributed to the overall reduction observed.

The impact of the intervention on anaemia and Hb levels

There were no significant differences in prevalence of anaemia or in Hb levels between children in the intervention group compared to children in the control group over the two years follow-up period. However, overall, there was significant improvement in mean Hb levels and a reduction in prevalence of anaemia. The observed changes in the prevalence of anaemia and Hb levels followed a similar trend as changes in the prevalence and infection intensity of malaria suggesting that malaria infection was the most important contributor to anaemia in the studied population in line with findings of other studies (Carneiro *et al*, 2006; Enhardt *et al*, 2006). Further, the antihelmintic intervention might have contributed to the improvement in Hb levels and hence reduction in the prevalence of anaemia by acting through reductions in prevalence and infection intensity of helminth infections (*S. haematobium*, *S. mansoni* and hookworms) in line with reports by other investigators (Beasley *et al*, 1999; Koukounari *et al*, 2007; Tohon *et al*, 2008). This argument is supported by baseline findings (Chapter 3) whereby multivariate analysis showed *P. falciparum* and *S. haematobium* infections as the important predictors of anaemia. As the overall prevalence of *P. falciparum* and *S. haematobium* infections decreased from baseline levels of 29.8% and 19.7% to 24.5% and to 3.6%, respectively, the prevalence of anaemia droped from baseline levels of 34.4% to 13.9% at the end of the intervention showing that *P. falciparum* and *S. haematobium infections* were the dominant contributors to anaemia. Other studies which have provided further evidence on the impact of malaria infections on anaemia include studies on interventions directed against malaria such as Insecticide Treated Nets (ITNs) and chemoprophylaxis. These studies have demonstrated increased Hb levels and a strong impact on anaemia (Schellenberg *et al,* 2001; Shulman *et al*, 1999). The most striking impact of the antihelmintic intervention was on severe anaemia which was reduced by 100% in all groups. Many studies have described the relationship between anaemia and infection with one or more parasitic infections such as malaria (Stephenson *et al*, 1985; Anumudu *et al*, 2009; Muhangi *et al*, 2007; Rogerson *et al*, 2000; Rogerson, 2006; Carneiro *et al*, 2006; Pradhan 2009 and Bouyou-Akotet *et al*, 2009). In children aged between one and four years in malaria endemic countries, anaemia is the most common clinical manifestation of malaria (Murphy *et al*, 2001). Several studies have shown a relationship between schistosomiasis, STH infections and anemia (Latham *et al*, 1983; Sturrock *et al*, 1996; Muhangi *et al*, 2007; Mupfasoni *et al*, 2009; Tohon *et al*, 2008; Stephenson *et al*, 1985; Lwambo *et al*, 2000; Beasley *et al*, 1999; Koukounari *et al* 2006; Ezeamama *et al*, 2008 and Guyatt *et al*, 2001) and supports the findings of our study where the prevalence of anaemia decreased significantly at the second round of follow up after significant reductions in both the prevalence and intensity of malaria and *S. hematobium* infections. In a study by Guyatt *et al* (2001) in Tanga, Tanzania it was shown that *S. haematobium* in addition to

hookworm infection were important predictors of anaemia. *S. haematobium* and hookworm contributed about 15% and 6% of all anaemia cases, respectively in the study population and following deworming with albendazole and praziquantel, the prevalence of anaemia was reduced by 25% while that of moderate to severe anaemia was reduced by 50%. A study in Nigeria demonstrated that anaemia was more frequent in children infected with *S. haematobium* and in those infected with *P. falciparum* than in uninfected children (Tohon *et al* 2008). Other studies which examined the relationship between *P. falciparum* infection and anaemia in children were that of Bouyou-Oktet *et al* (2006) in Gabon and that of McElroy *et al* (2000) in western Kenya. The two studies demonstrated strong association between the two whereby malaria parasitaemia was negatively correlated with Hb levels and was identified as the major risk factor for childhood anaemia, a finding which concurs with findings of the current study. Importantly, a decrease in malaria infection (prevalence, malaria parasite density and number of observed malaria attacks) and an increase in Hb levels were observed at the end of the intervention. Although the increase in Hb levels could be the result of the antihelmintic intervention on *S. mansoni*, *S. haematobium* and hookworm, a decline in malaria transmission in the current study may not be directly attributed to antihelmintic intervention. Other factors such as of seasonal variation due to climate and/or environmental factors may play a role as it has been previously mentioned.

The impact of the intervention on the prevalence and infection intensity of schistosomiasis and STH, multiple parasite infections and on different morbidity indicators

As expected, there was a significant reduction in the prevalence and infection intensity of schistosmiasis and STH infections over the two years of the antihelmintic intervention confirming the efficacy of antihelmintics praziquantel and albendazole in the treatment of schistosomiasis and STH infections (Saathhoff *et al*, 2004; Tohon *et al*, 2008; Bhargava *et al*, 2003). For *S. mansoni* infections, there was more than 50% reduction in the prevalence of moderate to heavy infections with significant differences between groups while for *S. haematobium* infections, there was 100% reduction in the prevalence of heavy infections for all groups. Further, children in the intervention group had significantly lower prevalence of *S. mansoni*, *S. haematobum* and hookworms compared to children in the control group over the two follow up years showing that repeated treatments have more impact on prevalence of infection compared to a single annual dose. For *S. haematobium* infection, there were significant differences in infection intensity between children in the intervention group compared to the control group. No significant differences in infection intensities were observed between children in the intervention and control group for *S. mansoni* and hookworm infections. Overall, the reduction in prevalence and infection intensity of *S. mansoni* infection was lower compared to what was observed for *S. haematobium* and hookworm infection (where a reduction of 100% was achieved) even for the control group. This could mean that praziquntel is more effective against *S. haematoium* compared to *S. mansoni* infection. Another possible explanation could be the existence of different transmission patterns for *S. mansoni* and *S. haematobium* in the area. Since *S. mansoni* infection is acquired through contact with cercaria in lake waters, it is most likely to occur throughout the year or that *S. mansoni* had a faster re-infection rates. *S. haematobium* infection is acquired through contact with cercaria in streams, ponds and paddy fields and is most likely to occur mainly during the rain season. The latter explanation is in line with findings of a study in Mozambique (Augusto *et al*, 2009) which observed that the outcome of chemotherapy for *S. haematobium* infection depends on timing of treatment relative to the annual transmission season. The study observed that the effect of praziquantel treatment (reductions in prevalence, infection intensity and improved cure rate) against *S. haematobium* infection is enhanced if treatment is done during the low transmission season. In Mwanza region the high transmission season for *S. haematobium* lasts for approximately four to five months between April and August each year and coincides with a period of high infection rates in snails (Webbe, 1962;

Lwambo, 1988). During the current study, treatment was done durint the end of the dry season between October to November each year which is the low transmission season in the area. It is therefore possible that timing of treatment relative to transmission season resulted into the observed higher reductions in prevalence and infection intensity for *S. haematobium* compared to *S. mansoni*. The public health implication of this observation is that while timing of single dose treatment in relation to transmission season of *S. haematobium* (and hookworm) infection may be important, repeated treatments of more that once a year may be necessary in areas with high transmission of *S. mansoni* infection in order to achieve significant reductions in prevalence, infection intensities and related pathology.

These results show that the two years of antihelmintic treatment with praziquantel lead to complete resolution of *S. haematobium* related morbidity in line with reductions in prevalence and infection intensity of *S. haematoium* infection as discussed previously. This is a clear confirmation of the strong efficacy of praziquantel on *S. haematobium* infection as reported by other investigators (Tohon *et al* 2008; Garba *et al*, 2004; Campagne *et al*, 2001; Laurent *et al*, 1990; Hatz *et al* 1998; King *et al*, 1988). Treatment using praziquantel at a dosage rate of 40mg/kg is known to bring about clearance of urinary tract pathology within 6 months after treatment (Sacko, 2006; Hatz *et al* 1998; Hatz *et al*, 1990; Richter, 2003). On the other hand, except for periportal fibrosis and enlarged portal vein diameter (PVD), overall ultrasound detected morbidity related to *S. mansoni* infections i.e. hepatomegaly, splenomegaly, and hepatosplenomegaly persisted after the two years of the intervention (Table 5.7). This can be explained by the fact that although there was marked reduction in the prevalence and infection intensity of *S. mansoni*, about 20% of the infections persisted, half of which were moderate to severe infections. This persistent infection after the two years of the intervention might have contributed to non-resolution of *S. mansoni* related pathology. Another explanation could be that morbidity due to intestinal schistosomiasis regresses slowly after treatment particularly in areas of intense transmission compared to morbidity due to urinary schistosomiasis as observed by previous studies (Richter *et al* 2003; Mohamed *et al*., 1991; Doehring-Schwerdtfeger *et al*., 1992; Frenzel *et al*., 1999; Kabatereine *et al*, 2004). Furthermore, the morbidity which did not show significant resolution (hepatomegaly, splenomegaly, and hepatosplenomegaly) can be caused by both *S. mansoni* and malaria, the prevalence and infection intensity of which persisted after the two years of the antihelmintic intervention. Involvement of both *S. mansoni* and malaria in hepatosplenic disease has already been described by previous studies (Booth *et al*, 2004a; Mwatha *et al*, 2003), and it has been shown that malaria infection can delay or cause non-complete resolution of morbidity due to *S. mansoni* infection following treatment with praziquantel (Vennervald *et al*, 2004; Vennervald *et al*, 2005; Booth *et al*, 2004b). The public health implication of these results is that in areas where both *S. mansoni* and *P. falciparum* malaria are co-endemic as is the case of the current study, malaria infection can complicate resolution of morbidity related to intestinal schistosomiasis following praziquantel treatment. The observed reductions in overall prevalence of multiple parasitic infections (with significant differenced between groups) corresponds to reductions in prevalence and infection intensity of individual parasite species as discussed previously.

In conclusion, findings of the current study show that in areas co-endmic for malaria and helminth infections, an antihelmintic intervention may not have a direct impact on malaria infection (prevalence, parasite density and frequency of malaria attacks). However, the intervention have an impact in terms of reductions in prevalence and infection intensity of single and multiple infections and on overall diasease morbidity caused by co-infections such as anaemia and hepatosplenomegaly. This in turn indicate that if integrated control of parasitic infections in school and pre-school children is adopted, there would much benefits in terms of reductions in overall disease burden than what would be observed if a single disease approach is used.

5.5. References

Anumudu, C.I., Okafor, C. M., Ngwumohaike, V., Afolabi, K. A., Nwuba, R. I., Nwagwu, M. (2007). Epidemiological factors that promote the development of severe malaria anaemia in children in Ibadan. *Afr Health Sci,* **7** (2): 80-85.

Augusto, G., Magnussen, P., Kristensen, T. K., Appleton, C. C., Vennervald, B. J. V. (2009). The influence of transmission season on parasitological cure rates and infection intensity after praziquantel treatment of *Schistosoma haematobium* infected school children in Mozambique. *Parasitology,* **136:** 1771-1779.

Beasley, N. M., Tomkins A. M., Hall, A., Kihamia, C. M., Lorri, W. (1999). The impact of population level deworming on the haemoglobin levels of school children in Tanga, Tanzania. *Trop. Med. Intl. Health,* **4**: 744-750.

Bhargava, A., Jukes, M., Lambo, J., Kihamia C. M., Lorri, W. (2003). Anthelmintic treatment improves the haemoglobin and serum ferritin concentrations of Tanzanian school children. *Food. Nutr. Bull,* **24**: 332-342.

Booth, M., Vennervald, B. J., Kenty, L., Butterworth, A. E., Kariuki, H. C., Kadzo, H., Ireri, E., Amaganga, C., Kimani, G., Mwatha, J. K., Otedo, A., Ouma, J. H., Muchiri, E., Dunne, D. W. (2004a). Micro-geographical variation in exposure to *Schistosoma mansoni* and malaria, and exacerbation of splenomegaly in Kenyan school-aged children. *BMC Infect Dis,* **17**; 4: 13.

Booth, M., Vennervald, B. J., Butterworth, A. E., Kariuki, H. C., Amaganga, C., Kimani, G., Mwatha, J. K., Otedo, A., Ouma, J. H., Dunne, D. W. (2004b). Exposure to malaria affects the regression of hepatosplenomegaly after treatment for Schistosoma mansoni infection in Kenyan children, *BMC Med,* **27**: 2: 36.

Bouyou-Akotet, M. K., Dzeing-Ella, A., Kendjo, E., Etoughe, D., Ngoungou, E. B., Planche, T., Koko, J., Kombila, M. (2009). Impact of *Plasmodium falciparum* infection on the frequency of moderate to severe anaemia in children below 10 years of age in Gabon. *Mal J,* **8**: 166.

Brutus, L., Laurence, Watier., Briand, V., Hanitrasoamampionona, V., Razanatsoarilala, H., Cot, Michel (2006). Parasitic co-infections. Does Ascaris lumbricoides protect against plasmodium falciparum infection? *Am. J. Trop. Med. Hyg,* **75** (2): 194-198.

Brutus, L., Watier, L., Hanitrasoamampionona, V., Razanatsoarilala, H., Cot, M. (2007). Confirmation of the protective effect of Ascaris lumbricoides on *Plasmodium falciparum* infection: Results of a randomized trial in Madagascar. *Am. J. Trop. Med. Hyg.* **77** (6): 1091-1095.

Chitsulo, L., Engels, D.; Montressor, A.; Savioli, L. (2000). The global status of schistosmiasis and its control. *Acta Trop,* **77** (1): 41-51.

Campagne, G., Garba, A., Bakire, H., Vera, C., Sidiki, A. (2001). Continued ultrasonographic follow up of children infected with *schistosoma haematobium* after treatment with praziquantel. *Trop. Med. Int Health,* **6**: 24-30.

Carneiro, I. A., Smith, T., Lusingu, J. P., Malima, R., Utzinger, J., Drakeley, C. J. (2006). Modeling the relationship between the population prevalence of Plasmodium falciparum malaria and anemia. *Am. J. Trop. Med. Hyg,* **75** (2 Suppl): 82-89.

De Silva, NR, Brooker, S, Hotez, P. J., Montresor, A., Engels, D., Savioli, L. (2003). Soil transmitted helminth infections: Updating the global picture. *Trends Parasitol*, **19**: 547-551.

De Silva, N. R. (2003). Impact of mass chemotherapy on the morbidity due to soil transmitted nematodes. *Acta Trop*, **86**: 197-214.

Dreyfuss, M. L., Stoltzfus, R. J., Shrestha, J. B., Pradhan, E. K., Leclerg, S. C., Khatri, S. K., Shresha, S. R., Katz. J., Albonico, M., West, K. P. (2000). Hookworms, malaria and vitamin A deficiency contributes to anaemia and iron deficiency among pregnant women of Nepal. *J Nutr,* **130**: 2527-2536.

Dondorp, A. M., Angus, B. J., Chotivanich, K., Silamut, K., Ruangveerayuth, R., Hardeman, M. R., Kager, P. A., Vreeken, J., White, N. J. (1999). Red blood cell deformability as a predictr of anaemia in severe falciparum malaria. *Am. J. Trop. Med. Hyg*, **60**: 733-737.

Doehring-Schwerdtfeger E., Abdel-Rahim, I. M., Kardorff, R., Kaiser, C., Franke, D., Schlake, J., Richter, J., Elsheikh, M., Mohamed-Ali, Q., Ehrich, J. H. (1992). Ultrasonographical investigation of periportal fibrosis in children with *Schistosoma mansoni* infection: reversibility of morbidity twenty-three months after treatment with praziquantel. *Am. J. Trop. Med. Hyg*, **46** (4): 409-415.

Druilhe, P., Tall, A., Sokhna, C. (2005). Worms can worsen malaria. Towards a new means to roll back malaria? *Trends Parasitol*, **21:** 359-362.

Ezeamama, A. E., Friedman, J. F., Olveda, R. M., Acosta, L. P., Kurtis, J. D., Mor, V., McGarvey., S. T. (2005). Functional significance of low intensity polyparasite helminth infections in anaemia. *J. Inf. Dis*, **192:** 2160-2170.

Ezeamama, A. E., Friedman, J. F., Acosta, L. P., Bellinger, D. C., Langdon, G. C., Manalo, D. L., Olveda, R. M., Kurtis, J. D., McGarvey, S. T. (2005). Helminth infection and cognitive impairment among Filipino children. *Am J Trop Med Hyg,* **72** (5): 540-548.

Ezeamama, A. E., McGarvey, S. T., Acosta, L. P., Zierler, S., Manalo, D. L., Wu, H. W., Kurtis, J. D., Mor, V., Olveda, R. M., Friedman, J. F. (2008). The synergistic effect of concomitant schistosomiasis, hookworm, and trichuris infections on children's anemia burden. *PLoS Negl Trop Dis*, **2** (6): e245.

Enhardt, S., Burchard, G. D., Mantel, C., Cramer, J. P., Kaiser, S. (2006). Malaria, anaemia and malnutrition in African school children. Defining intervention strategies. *J. Inf. Dis,* **194**: 108-114.

Ekval, H .(2003). Malaria anaemia. *Curr. Opin. Haemat,* **10**: 108-114.

Facer., C. A., Bray., R. S., Brown, J. (1979). Direct comb antiglobulin reactions in Gambian children with plasmodium falciparum malaria. I. Incidence and class specificity. *Clin. Exp. Immunol*, **35**: 119.

Frenzel, K., Grigull L., Odongo-Aginya E., Ndugwa C. M., Loroni-Lakwo T., Schweigmann U., Vester U., Spannbrucker N., Doehring, E. (1999). Evidence for a long-term effect of a single dose of praziquantel on *Schistosoma mansoni*-induced hepatosplenic lesions in northern Uganda. *Am. J. Trop. Med. Hyg,* **60** (6): 927-931.

Gallagher, M., Malhotra I., Mungai P. L., Wamachi A. N., Kioko J. M., Ouma J. H., Muchiri E., King C. L. (2005). The effects of maternal helminth and malaria infections on mother-to-child HIV transmission. *AIDS*, **19** (16): 1849-1955.

Garba, A., Campagne, G., Tassie, J. M., Barkire, A., Vera, C. (2004). Long term impact of mass treatment by praziquantel on morbidity due to *schistosoma haematobium* in two hyperendemic villages of Niger. *Bull Soc. Pathol Exot,* **97**: 7-11.

Guyatt, H. L., Brooker S., Kihamia C. M., Hall A., Bundy D. A. (2001). Evaluation of efficacy of school-based anthelmintic treatments against anaemia in children in the United Republic of Tanzania. *Bull World Health Organ, 2001*; **79** (8):695-703.

Hatz, C., Mayombana, C., de Savigny D., Macpherson, C., Koella, J., Degremont, A., Tanner M. (1990). Ultrasound scanning for detecting morbidity due to *Schistosoma haematobium* and

its resolution following treatment with different doses of praziquentel. *Trans Roy. Soc. Trop. Med, Hyg*. **84**: 84-88.

Hatz, CF., Vennervald, B. J., Nkulila, T., Vounatsou, P., Kombe, Y., Mayombana, C., Mshinda, H., Tanner, M. (1998). Evolution of *Schistosoma haematobium*-related pathology over 24 months after treatment with praziquantel among school children in southeastern Tanzania.. *Am J Trop Med Hyg,* **59** (5): 775-781.

Helmby, H., Kullberg, M., Troye-Blomberg, M. (1998). Altered immune responses in mice with concomitant *Schistosoma mansoni* and *Plasmodium chabaudi* infections. *Infect Immun,* **66** (11): 5167-5174.

Hotez, P. J., Brooker, S., Bethony, J. M. (2004). Current concepts: Hookworm infection. *N Engl J Med,* **351** (8), 799-808.

Kabatereine, N. B., Kemijumbi J., Ouma J. H., Kariuki H. C., Richter J., Kadzo H., Madsen H., Butterworth A. E., Ørnbjerg N., Vennervald B. J. (2004). Epidemiology and morbidity of *Schistosoma mansoni* infection in a fishing community along Lake Albert in Uganda. *Trans Roy Soc Trop Med Hyg*, **98** (12): 711-718.

Koukounari, A., Fenwick, A., Whawell, S., Kabatereine, N. B., Kazibwe, F., Tukahebwa, E. M., Stothard, J. R., Donnelly, C, A., Webstar, J. P. (2006). Morbidity indicators of schistosomma mansini: Relationship between infection and anaemia in Ugandan school children before and after praziquantel and albedazole chemotherapy. *Am. J. Trop. Med. Hyg*, **75** (2): 278-286.

King, C. H., Lombardi G., Greenblatt, R., Hodder S., Kinyanjui H., Ouma, J. H., Odiambo, O., Bryan P. J., Muruka J., Magak, P., Weinert D., Ransohoff, D., Houser, H., Koech, D., Arap Siongok, T. K., Mahmood A. A. F. (1988). Chemotherapy based control of schistosomiasis haematobia. Metrifonate versus praziquantel in the control of intensity and prevalence of infection. *Am. J. Trop. Med. Hyg,* **39**: 295-305.

Latham, M. C., Stephenson, L, S., Hall, A., Wolgemuth, J. C., Elliot, t. C., Crompton, D. W (1983). Patasitic infections, anaemia and nutritional stautus: A study of their interrelationship and the effect of prophylaxis and treatment on workers in kwale district, Kenya. *Trans. Roy. Soc. Trop. Med. Hyg,* **77** (1): 41-48.

Laurent, C., Lamothe, P., Develoux M., erin, B., Mouchet, F. (1990). Ultrasonophic asessment of urinary tract lesions due to *schistosoma haematobium* in niger after four consecutive years of treatment with praziquantel. *Trop. Med. Parasitol,* **41**: 139-142.

Le Hesran, J. Y., Akiana, J., Ndiaye, E. H. M., Dia, M., Senghor, P., konate, L. (2004). Severe malaria attcch is associetsd with high prevalence of ascaris lumbricoides infection among children in rural Senegal. *Trans. Roy. Soc. Trop Med. Hyg*, **98**: 397-399.

Lwambo, N. J., Broker S., Siza, J. E., Bundy D. A., Guyatt, H. (2000). Age patterns in stunting and anaemia in African school children: A cross sectional study in Tanzania. *Eur. J. Clin. Nutr,* **54**: 36-40.

Lwambo, N. J. (1988). Transmission of urinaty schistosomiasis in Sukumaland, Tanzania: Snail infection rates and incidence in school children. *J Helminthol,* **62** (3): 213-217.

Maizels R. M., Balic A., Gomez-Escobar N., Nair M., Taylor, M. D., Allen J. E (2004). Helminth parasites–masters of regulation. *Immunol Rev,* **201***:* 89–116.

McElroy, P. D., ter Kuile F. O., Lal A. A., Bloland P. B., Hawley W. A., Oloo A. J., Monto A. S., Meshnick S. R., Nahlen B. L. (2000). Effect of *Plasmodium falciparum* parasitemia density on hemoglobin concentrations among full-term, normal birth weight children in western Kenya, IV. The Asembo Bay Cohort Project. *Am J Trop Med Hyg*, **62** (4): 504-512.

Mohamed-Ali Q., Doehring-Schwerdtfeger E., Abdel-Rahim I. M., Schlake J., Kardorff R., Franke D., Kaiser C., Elsheikh M., Abdalla M., Schafer P. (1991). Ultrasonographical

investigation of periportal fibrosis in children with *Schistosoma mansoni* infection: reversibility of morbidity seven months after treatment with praziquantel. *Am J Trop Med Hyg,* **44** (4): 444-451.

Muhangi, L., Woodburn P., Omara M., Omoding N., Kizito D., Mpairwe H., Nabulime J., Ameke C., Morison L. A., Elliott A. M. (2007). Associations between mild-to-moderate anaemia in pregnancy and helminth, malaria and HIV infection in Entebbe, Uganda. *Trans Roy Soc Trop Med Hyg*, **101** (9): 899-907.

Mupfasoni, D., Karibushi, B., Koukounari, A., Ruberanziza, E., Kaberuka, T., Kramer, M. H., Mukabayire, O., Kabera, M., Nizeyimana, V., Deville, M. A., Ruxin, J., Webster, J. P., Fenwick, A. (2009). Polyparasite helminth infections and their association to anaemia and undernutrition in Northern Rwanda. *PLoS Negl Trop Dis*, **3** (9): e517.

Murray, J., Murray, A., Murray, M., Murray, C. (1998). The biological suppression of malaria: an ecological and nutritional interrelationship of a host and two parasites. *Am J Clin Nutr,* **31** (8): 1363-1366.

Murphy, S. C., Breman, J. G. (2001). Gaps in the childhood malaria burden in Africa: cerebral malaria, neurological sequelae, anemia, respiratory distress, hypoglycemia, and complications of pregnancy. *Am J Trop Med Hyg,* **64**: 57 - 67.

Mwangi, T. W., Bethony J. M., Brooker, S. (2006). Malaria and helminth interactions in humans: An epidemiological viewpoint. *Ann. Trop. Med. Parasitol,* **100** (7): 551-570.

Mwatha, J. K., Jones F. M., Mohamed G., Naus C. W., Riley E. M., Butterworth A. E., Kimani G., Kariuki C. H., Ouma J. H., Koech D., Dunne D. W. (2003). Associations between anti-*Schistosoma mansoni* and anti-*Plasmodium falciparum* antibody responses and hepatosplenomegaly, in Kenyan schoolchildren. *J Infect Dis*, **187** (8): 1337-1341.

Nacher, M., Singhasivanon, P., Yimsamran, S., Manibunyong, W., Thanyavanich, N., Wuthisen, P., Looareesuwan, S. (2002). Intestinal helminth infections are associated with increased incidence of *Plasmodium falciparum* malaria in Thailand. *J Parasitol,* **88** (1): 55-58.

Pradhan, P. (2009). Malarial anaemia and nitric oxide induced megaloblastic anaemia: a review on the causes of malarial anaemia. *J Vector Borne Dis,* **46** (2): 100-108.

Richter, J. (2003). The impact of chemotherapy on morbidity due to schistosomiasis. *Acta Trop,* **86** (2-3): 161-83.

Roberts, D. J., Pascual, C., Weatheral, D. J. (2005). The clinical and pathological features of malaria anaemia. *Curr. Trop. Microbiol. Immunol*, **295**: 137-167.

Rogerson, S. J., Van den Broek, N. R., Chaluluka, E., Qongwane, C., Mhango, C. G., Molyneux, M. E. (2000). Malaria and anemia in antenatal women in Blantyre, Malawi: a twelve-month survey. *Am J Trop Med Hyg*, **62** (3): 335-340.

Rogerson, S. (2006). What is the relationship between haptoglobin, malaria, and anaemia? *PLoS Med*, **3** (5): e200.

Saathoff, E., Olsen, A., Magnussen, P., Kvalsvig, J. D., Becker, W., Appleton, C. C. (2004). Patterns of *Schistosoma haematobium* infection, impact of praziquantel treatment and re-infection after treatment in a cohort of schoolchildren from rural KwaZulu-Natal/South Africa. *BMC Infect Dis,* **4**: 40.

Sacko, M. (2006). Assessment of the effect of two different treatment strategies on the morbidity caused by schistosoma haematobium infection among school aged children in the Niger River Basin, Mali. PhD Thesis, Faculty of Science, University of Copenhagen, Denmark.

Schellenberg, D., Menendez, C., Kahigwa, E., Aponte, J., Vidal, J., Tanner, M., Mshinda, H., Alonzo, P. L. (2001). Intermittent treatment for malaria and anaemia control at time of routine vaccination in Tanzanian infants: A randomized placebo controlled trial. *Lancet,* **357**: 1471-1477.

Shulman, C. E., Dorman, E. K., Kutts, F., Kawuondo, K., Bulmer, J. N., Peshu, N., Marsh, K. (1999). Intermittent sulphadoxine-pyrimethamine to prevent severe anaemia secondary to mlaria in pregnancy: A randomised placebo controllled trial. *Lancet*, **353:** 632-636.

Sokhna, C., Le Hesran, J. Y., Mbaye, P. A., Akiana, J., Camara, P., Diop, M., Ly, A., Druilhe, P. (2004). Increased malaria attacks among children presenting concomitant infections by *S. mamsoni* in Senegal. *Mal J*, **3**: 43.

Spiegel, A., Tall, A., Raphenon, G., Trape, J. F., Druilhe, P. (2003). Increased frequency of malaria attacks in subjects co-infected by intestinal worms and *Plasmodium falciparum* malaria. *Trans. Roy. Soc. Trop. Med. Hyg,* **97**: 198-199.

Stephenson, L. S., Latham, M. C., Kurz, K. M., Kinoti, S. N., Oduori, M. L., Crompton, D. W. T. (1985). Relationship of *Schistosoma haematobium*, hookworm and malarial infections and metrifonate treatment to haemoglobin levels in Kenyan school children. *Am J. Trop Med. Hyg*, **34** (3): 519-528.

Stephenson, L. S., Latham, M. C., Kinoti, S. N., Pertet, A. (1993). Physical fitness, growth and appetite of Kenyan school children with hookworm, T*richuris trichiura and Ascaris lumbricoides* infections are improved four months after a single dose of albendazole. *J Nutr*, **123:** 1036-1046.

Stephenson, L. S., Latham, M . C., Ottesen, E. A. (2000). Malnutrition and parasitic helminth infections. *Parasitol*, **121** (Suppl): S23-S38.

Stephenson, L. S. (1987). Impact of helminth infections on human nutrition. London and Philadelphia, Taylor and Francis Ltd.

Sokhna, C. (2004). Increase of malaria attacks among children presenting concormittant infections by Schistosoma mansoni in Senegal. *Mal J*, **3:** 43.

Sturrock, R. F., Thiong'o., F. W., Gachare, J. W., Omondi, B. G., Ouma, J. H., Mbugua, G., Butterworth, A, E. (1996). *Schistosomiasis mansoni* in Kenya. Relatioship between infection and anaemia in school children at the commnunti level. *Trans. Roy. Soc. Trop. Med. Hyg,* **90:** 48-54.

Tschikuka, J. G., Scott, M. E., Gray-Donald, K., Kalumba, O. N. (1996), Multiple infection with plasmodium and helminths in communities of low and relatively high socio-economic status. *Ann Trop Med Parasitol*, **90 (**3): 277-293.

Tohon, Z. B., Mainassara, H. B., Garba, A., Mahamane, A. E., Bosque-Oliva, E., Ibrahim, M., Duchemin, J. B., Chanteau, S., Boisier, P. (2008). Controlling schistosomiasis: Significant decrease of anaemia in Nigerian schoolchildren. *PLoS Negl Trop Dis,* **2** (5): e241.

Utzinger., J and Keiser, J. (2004). Schistosomiasis and Soil transmitted helminthiasis: Common rugs for treatment and control. *Expert opin. Pharma,* **5**: 263-285.

Vennervald, B. J. and Dunne, D. W. (2004). Morbidity in schistosomiasis: an update. *Curr Opin Infect Dis*, **17** (5): 439-447.

Vennervald, B. J., Booth, M., Butterworth, A., E, Kariuki, H. C., Kadzo H., Ireri E., Amaganga C., Kimani, G., Kenty, L., Mwatha, J., Ouma, J. H., Dunne, D. W. (2005). Regression of hepatosplenomegaly in Kenyan school-aged children after praziquantel treatment and three years of greatly reduced exposure to Schistosoma mansoni. *Trans Roy Soc Trop Med Hyg*, **99** (2): 150-60.

Waknine-Grinberg, J. H., Gold, D., Ohayon, A., Flescher, E., Heyfets, A., Doenhoff, M. J., Schramm, G., Haas, H., Golenser, J. (2010). Schistosoma mansoni infection reduces the incidence of murine cerebral Malaria**.** *Mal J,* **9**: 1186.

Webbe, G. (1962). The transmission of Schistosoma haematobium in an area of Lake Province, Tanganyika. *Bull World Health Org*, **27:** 59-85.

WHO (2000). Current global malaria situation. In: WHO expert committee on malaria. *WHO Technical Report Series 892, WHO, Geneva,* 2000. pp 5-6.

WHO (2002). Prevention and control of schistosomiasis and soil-transmitted helminthiasis. Report of WHO Expert Committee. *WHO technical Report Series 912*, WHO, Geneva, 2002, pp 1-29.

Weatheral, D. J., Abdalla, S. (1982). The anaemia of plasmodium falciparum malaria. *Br. Med. Bull*, **38:** 147-151.

Chapter 6: The impact of two anthelmintic treatment approaches on *P. falciparum* specific antibody responses among school and preschool children in Magu district, Tanzania

Abstract

A study was conducted to examine possible interactions between schistosome and STH infections and the effect of an antihelmintic intervention on *P. falciparum* specific antibody responses among school and pre-school children in Magu district, Mwanza region, Northwestern Tanzania. A total of 2822 serum samples were prepared from blood collected from 1572 children (baseline survey), 658 children (first follow up survey) and 592 children (second follow up survey). The immune response against *P. falciparum* infection was measured by determination of the level of IgG3 against *P. falciparum* schizont antigen (PfSE-IgG3) using the Enzyme Linked Immunosorbent Assay (ELISA) method. Out of 1505 children with complete baseline information, 1247 (82.9%) were seropositive for PfSE-IgG3. The seroprevalence of PfSE-IgG3 increased with age (χ^2 = 37.59, p < 0.001) and differed significantly among schools (χ^2 = 70.30, p<0.001). Geometric mean PfSE-IgG3 levels increased with age (F = 35.92, p = 0.001) and differed significantly among schools (F = 25.72, p<0.001). The seroprevalence of PfSE-IgG3 was significantly higher in children infected with *P. falciparum* (χ^2 = 41.92, p<0.001), *S. haematobium* (χ^2 = 8.74, p<0.01) and hookworm (χ^2 = 23.10, p<0.001) compared to children without any infection. Children with co-infections of *P. falciparum* and *S. haematobium* had significantly higher levels of PfSE-IgG3 responses compared to uninfected children (t = 5.52, p<0.001) or children with *P. falciparum* infection only (t = 2.67, p<0.01). Children co-infected with *P. falciparum* and hookworm had significantly higher levels of PfSE-IgG3 responses compared to uninfected children (t = 6.93, p<0.001) or children with *P. falciparum* infection only (t = 2.75, p<0.01). The seroprevalence and levels of PfSE-IgG3 were not associated with sex or *S. mansoni* infection. In a multivariate linear regression analysis, age group, *P. falciparum, S. haematobium* and hookworm infections were significant predictors of PfSE-IgG3 levels after adjusting for sex. PfSE-IgG3 levels were positively correlated with infection intensities of *P. falciparum*, *S. haematobium* and hookworm. For each parasite infection, PfSE-IgG3 levels increased with increasing infection intensity indicating possible interactions between PfSE-IgG3 and *P. falciparum, S. haematobium* and hookworm infections. PfSE-IgG3 levels were significantly associated with splenomegaly and hepatosplenomegaly. Levels of PfSE-IgG3 were higher in children with splenomegaly compared to those without splenomegaly (t = 12.78, p<0.001). Likewise, children with hepatosplenomegaly had significantly higher PfSE-IgG3 levels compared to children without hepatosplenomegaly (t = 24.24, p<0.001). In a multivariate logistic regression analysis PfSE-IgG3 was an important predictor of both splenomegaly and hepatosplenomegaly. Overall, there was a significant increase (t = 2.23, p = 0.027) in post-treatment geometric mean PfSE-IgG3 levels particularly for children with baseline age of 9 -13 years in the intervention group. In conclusion, this study has demonstrated that *P. falciparum* and *S. haematobium* co-infection and *P. falciparum* and hookworm co-infections are positively correlated with PfSE-IgG3 levels indicating positive interactions of *S. haematobium* and hookworm infections on anti-*P. falciparum* immune responses. Further, anthelmintic treatment of schistosome and hookworm infections was associated with increased levels of PfSE-IgG3 indicating a positive impact on anti-*P. falciparum* immune responses. However, it was not clear if this increase was associated with improved protection against *P. falciparum* infection and disease.

6.1. Introduction

There is increasing evidence showing that interactions exist between malaria and heminth infections in humans (Nacher *et al*, 2002; Brooker *et al*, 2006; Brooker *et al*, 2007; Mwangi *et al*, 2006). Several epidemiological and immunological studies have reported both positive and negative associations between malaria and helminth infections (Diallo *et al*, 2004; Lyke *et al*, 2006; Hartgers and Yazdanbackhsh, 2006; Druilhe *et al*, 2005). Other studies have reported the existence of cross-reactive epitopes between schistosome and *P. falciparum* antigens (Helmby, 2007; Naus *et al*, 2003). Meanwhile the nature and mechanisms of interactions in malaria-helminth co-infections and their effects on disease progression and outcome are not fully understood (Hartgers and Yazdanbackhsh, 2006; Lyke *et al*, 2006; Hartgers *et al*, 2009), many studies point at immunological modulation as the basis of the observed interactions arguing that chronic helminth infections modulate immune responses to *P. falciparum* infections from a Th1 mediated immune response to a predominantly Th2 mediated immune response (Gryzch *et al*, 1991; Maizels *et al*, 1993; Maizels *et al*, 2004; Spiegel *et al*, 2003). Whereas a Th1 immune response is characterised by production of pro-inflamatory cytokines such as IL-1β, IL-2, IL-6, IL-12, IFN-γ and TNF-α and cytophilic antibody subclasses such as IgG1 and IgG3 which are protective against malaria (Maizels *et al*, 1993; Good *et al*, 2005; Choudhurry, *et al*, 2000; Mohan *et al*, 1997; Artavanis-Tsakonas and Riley, 2002; Druilhe *et al*, 2005), a Th2 immune response is characterised by production of anti-inflammatory cytokines such as IL-4, IL-5, IL-10, IL-13, TGF-β and IgE and the non-cytophilic antibody subclasses such as IgG2, IgG4 and IgM which are not protective against malaria (Maizels, 1993; Maizels *et al*, 2004; Mosmann and Coffman, 1989; Spiegel *et al*, 2003). As a result of this immunomodulation, helminth infected individuals may have increased susceptibility to *P. falciparum* infection and hence at increased frequency of malaria attacks and severe disease (Nacher *et al*, 2002; Sokhna *et al*, 2004; Tschikuka *et al*, 1996; Egwunyenga *et al*, 2001; Lewinson *et al*, 1975). In line with helminth induced immunomodulation, there is evidence suggesting that treatment of schistosomiasis with praziquantel leads to altered anti-schistosome immune responses (Grogan *et al*, 1996; Satti *et al*, 1998; Mutapi *et al*, 1998; Mutapi *et al*, 2003) which in turn may lead to changes in the subclasses and levels of anti-*P. falciparum* antibody responses in co-infected individuals (Remoue *et al*, 2003; Diallo *et al*, 2004; Mutapi *et al*, 2003). There are few randomised community based prospective studies which have looked at immunological parameters in malaria and helminth co-infections in humans (Hartgers *et al*, 2009). The current study have examined the relationship between schistosome (*S. mansoni* and *S. haematobium*) and hookworm infections and *P. falciparum* specific antibody responses and the impact of an antihelmintic treatment intervention on *P. falciparum* specific antibody responses among school and pre-school children in Magu district, Mwanza region, Northwestern Tanzania.

6.2. Methodology

6.2.1. Study design and sample collection

The study was conducted in Magu district, Mwanza region, Northwestern Tanzania. The study comprised of a baseline survey which was conducted between October to November 2006 and two follow up surveys at 12 and 24 months after baseline. The first follow up survey was conducted between October to November 2007 while the second follow up survey was conducted between October to November 2008. Details regarding the study area and population, study design, sampling procedures, randomization and treatments are given in chapter 2. Blood samples were collected from all children who participated in the study from which serum was prepared.

6.2.2. Laboratory methods

Immediately after preparation, serum samples were preserved in a cool box (4^0C) and transported to the National Institute for Medical Research (NIMR), Mwanza Centre laboratory where they were preserved by deep freezing at -20^0C. The samples were latter packed in serum boxes, preserved in dry ice and transported to the DBL-Centre for Health Research and Development, University of Copenhagen, Denmark where they were preserved at -20^0C until the time when *P. falciparum* specific antibody ELISA was performed. The immune response against *P. falciparum* infection was measured by determination of the level of immunoglobulin G3 (IgG3) against *P. falciparum* schizont antigen (PfSE) using the Enzyme Linked Immunosorbent Assay (ELISA) method as described in detail in chapter 2. The specific antibody response for PfSE-IgG3 was read at dual wavelength of 490/595 and expressed as optical density (OD).

6.2.3. Data analysis

OD values for PfSE-IgG3 were exported from microplate manager version 5.2.1 (Bio-Rad Laboratories, Inc., USA) to Microsoft excel and then entered into STATA version 10 (STATA Cooperation, Texas, USA) for analysis. The PfSE-IgG3 data were normalised by log transformation (log(x+0.12)). The OD value of individual samples was obtained by subtracting the mean of the two blank for each plate. The positive cut of value was determined by taking the mean OD plus two standard deviations (mean + 2SD) of the negative controls resulting in a positive OD cut of value of 0.041. Comparison of PfSE-IgG3 seroprevalence between different exposure groups was done using the chi-square test. The student's t-test and one way analysis of variance (ANOVA) was used to compare geometric mean PfSE-IgG3 levels where two or more than two groups were compared, respectively. The t-test and ANOVA were performed on log transformed values of PfSE-IgG3. Geometric mean PfSE-IgG3 and their corresponding 95% confidence intervals (95% CI) were calculated using STATA version 10 for seropositive samples only. Correlation between PfSE-IgG3 levels and infection intensity of *P. faciparum, S. mansoni, S. haematobium* and hookworm infections were determined using linear regression method. Multiple linear regression analysis was also used to assess predictors of PfSE-IgG3 levels. Multiple logistic regression analysis was used to assess the relationship between splenomegaly/hepatosplenomegaly and PfSE-IgG3 levels. All graphs were drawn using MS-Excel and STATA version 10 as appropriate. A p-value of < 0.05 was considered statistically significant.

6.3. Results

6.3.1. Baseline distribution of PfSE-IgG3 responses

A total of 2822 serum samples were obtained in all three collection rounds i.e. baseline survey (1572 samples), first follow up survey (658 samples) and second follow up survey (592 samples). For the baseline study, serum samples from 1505 children with complete parasitological, ultrasound and PfSE-IgG3 data were included in the analysis of whom 741 (49.2%) were boys. Out of these 1247 (82.9%) were seropositive for PfSE-IgG3. The seroprevalence of PfSE-IgG3 differed significantly among age groups, being highest in older children (9-13 years) compared to young children (3-5 years) (73.5% vs 93.3%) ($\chi^2 = 37.59$, $p < 0.001$). Further, the seroprevalence of PfSE-IgG3 differed significantly among schools, being highest in Milambi primary school (95.8%) and lowest in Nyashimo primary school (71.8%) ($\chi^2 = 70.29$, $p < 0.001$). Geometric mean PfSE-IgG3 was 0.264 (95% CI 0.244-0.287) without significant differences between boys and girls ($t = -0.36$, $p = 0.716$). Geometric mean PfSE-IgG3 levels differed significantly among age groups ($F = 35.92$, $p < 0.001$)

whereby the highest geometric mean PfSE-IgG3 level (0.534, 95% CI 0.454-0.628) was observed in older children (9 – 13 years) while the lowest geometric mean PfSE-IgG3 levels (0.158, 95% CI 0.130-0.191) was observed in younger children (3 – 5 years). Geometric mean PfSE-IgG3 levels also differed significantly among schools ($F = 25.72$, $p < 0.001$).

6.3.2. Relationship between PfSE-IgG3 levels and infection status

The seroprevalence of PfSE-IgG3 was significantly higher in children infected with *P. falciparum* ($\chi^2 = 41.92$, $p < 0.001$), *S. haematobium* ($\chi^2 = 8.72$, $p < 0.01$) and hookworm ($\chi^2 = 23.10$, $p < 0.001$) compared to children without any infection. The seroprevalence of PfSE-IgG3 was not associated with sex or *S. mansoni* infection. In a multivariate logistic regression analysis, age group, *P. falciparum, S. haematobium* and hookworm infection were predictors of PfSE-IgG3 seroprevalence (table 6.1).

Table 6.1 Results of multivariate logistic regression analysis showing important predictors of PfSE-IgG3 seroprevalence with adjusted odds ratios and p-values (n = 1505).

Independent variable	Categories	Adjusted OR (95% CI)	P-Value
Age group	3 - 5 years	1	
	6 – 8 years	1.747 (1.278 – 2.387)	< 0.001
	9 – 13 years	4.445 (2.460 – 8.032)	< 0.001
P. falciparum infection	Negative	1	
	Positive	4.910 (3.161 – 7.629)	< 0.001
S. haematobium infection	Negative	1	
	Positive	1.60 (1.070 – 2.410)	0.022
Hookworm infection	Negative	1	
	Positive	4.941 (2.564 – 9.520)	< 0.001

Overall, significantly higher PfSE-IgG3 responses were detected in children with patent *P. falciparum, S. haematobium* and hookworm infections compared to children without any infection (table 6.2). There was no significant difference in PfSE-IgG3 responses between children infected with *S. mansoni* compared to un-infected children ($t = -0.89$, $p = 0.373$).

Table 6.2. Antibody responses (geometric mean PfSE-IgG3) in children with different parasitic infections by age group (n = 1505).

Infection status	**Geometric mean PfSE-IgG3 levels (OD) (95% CI)**			
	3 – 5 years	6 – 8 years	9 – 13 years	Overall
Not infected	0.079 (0.058-0.109)	0.185 (0.151-0.225)	0.356 (0.229-0.551)	0.152 (0.129-0.180)
P. falciparum only	0.248 (0.161-0.381)	0.428 (0.350-0.523)	0.718 (0.481-1.072)	0.388 (0.323-0.464)
S. mansoni only	0.169 (0.103-0.278)	0.150 (0.117-0.192)	0.418 (0.265-0.658)	0.178 (0.145-0.218)
S. haematobium only	0.373 (0.225-0.616)	0.256 (0.175-0.373)	0.638 (0.410-0.996)	0.316 (0.240-0.417)
Hookworm only	0.317 (0.150-0.668)	0.470 (0.346-0.638)	0.470 (0.120-1.834)	0.445 (0.341-0.580)
*P-value	< 0.001	< 0.001	0.297	< 0.001

***P-Values refer to differences in PfSE-IgG3 levels between different parasite infection status**

Children with coinfections of *P. falciparum* and *S. haematobium* had significantly higher levels of PfSE-IgG3 compared to uninfected children ($t = 5.52$, $p < 0.001$) or children with *P. falciparum* infection only ($t = 2.67$, $p < 0.01$). Likewise, children with co-infections of *P. falciparum* and hookworm produced higher levels of PfSE-IgG3 responses compared to uninfected children ($t = 6.93$, $p < 0.001$) or children with *P. falciparum* infection only ($t = 2.75$, $p < 0.01$) (table 6.3).

Table 6.3 Antibody responses (geometric mean PfSE-IgG3) in children with co-infections of *P. falciparum* and different helminth species (n = 1505).

Infection status	Geometric mean PfSE-IgG3 levels (OD) (95% CI)			
	3 – 5 years	6 – 8 years	9 – 13 years	Overall
Not infected	0.079 (0.058-0.109)	0.185 (0.151-0.225)	0.356 (0.229-0.551)	0.152 (0.129-0.180)
P. falciparum only	0.248 (0.161-0.381)	0.428 (0.350-0.523)	0.718 (0.481-1.072)	0.388 (0.323-0.464)
S. mansoni/P. falciparum	0.268 (0.131-0.545)	0.397 (0.302-0.521)	0.480 (0.282-0.817)	0.393 (0.314-0.492)
S. haematobium/P. falciparum	1.034 (0.512-2.088)	0.651 (0.437-0.968)	0.537 (0.166-1.730)	0.670 (0.482-0.932)
Hookworm/P. falciparum	0.394 (0.163-0.956)	0.633 (0.374-1.068)	1.370 (0.592-3.170)	0.613 (0.406-0.926)
*P-value	< 0.001	< 0.001	0.116	< 0.001

***P-Values refer to differences in PfSE-IgG3 levels between different infection status**

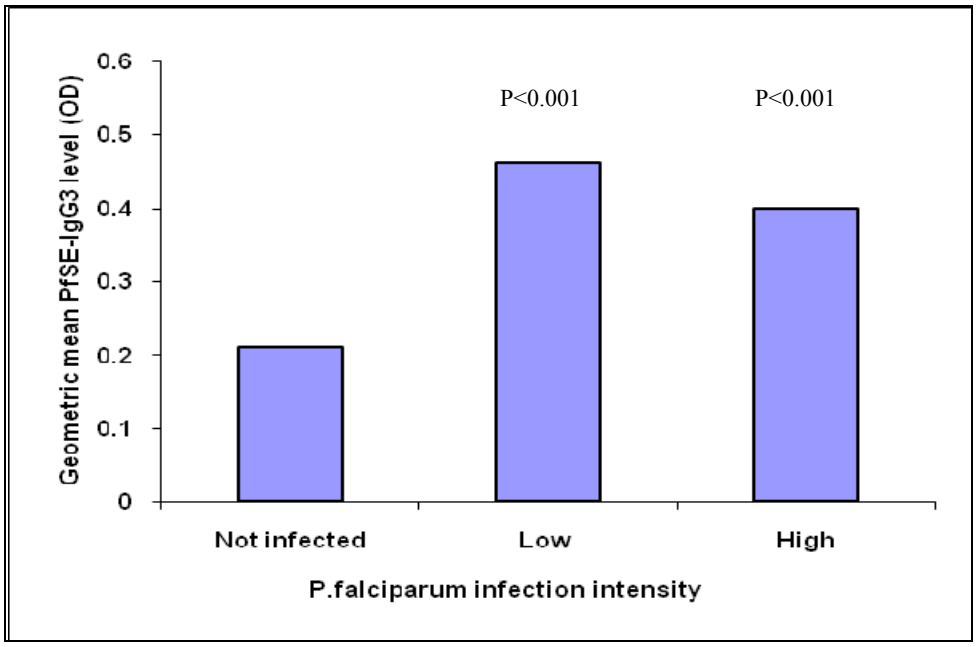
In a multivariate linear regression analysis, *P. falciparum* infection, *S. haematobium* infection, hookworm and age group were significant predictors of PfSE-IgG3 levels after adjusting for sex (table 6.4).

Table 6.4 Results of multivariate linear regression analysis showing predictors of PfSE-IgG3 levels (n = 1505).

Independent variable	β (95% CI)	P-Value
Age group	0.669 (0.492 – 0.847)	< 0.001
P. falciparum infection	1.104 (0.873 – 1.335)	< 0.001
S. haematobium infection	0.363 (0.096 – 0.630)	0.008
Hookworm infection	0.863 (0.570 – 4.277)	< 0.001

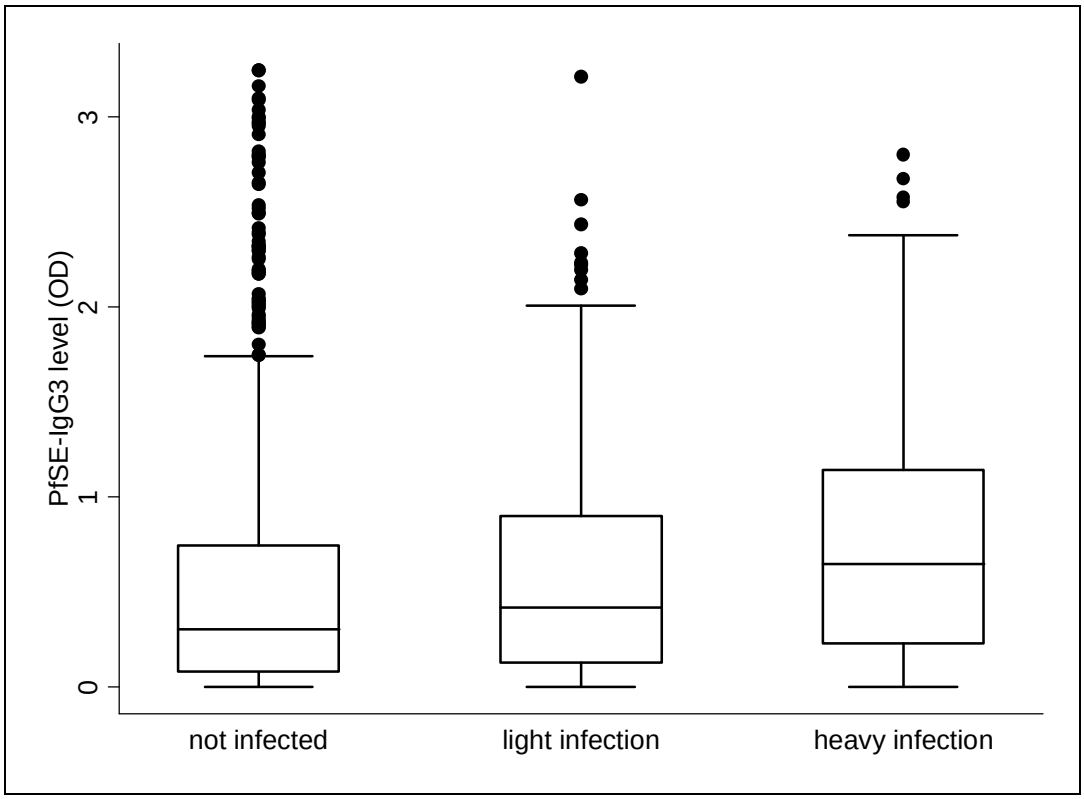
PfSE-IgG3 levels were positively correlated with infection intensity of *P. falciparum*, *S. haematobium* and hookworm indicating possible interactions between PfSE-IgG3 and *P. falciparum, S. haematobium* and hookworm infections. For *S. haematobium* and hookworm infections, PfSE-IgG3 levels increased with increasing infection intensity (Figures 6.2 – 6.3).

Figure 6.1 Bar chart showing the relationship between *P. falciparum* infection intensity categorised as not infected (n = 1056) low (n = 412) and high (n = 37) and PfSE-IgG3 levels.



Overall, PfSE-IgG3 levels were significantly higher in children with low and high *P. falciparum* infection intensity compared to un-infected children ($F = 41.12$, $p<0.001$) (Figure 6.1).

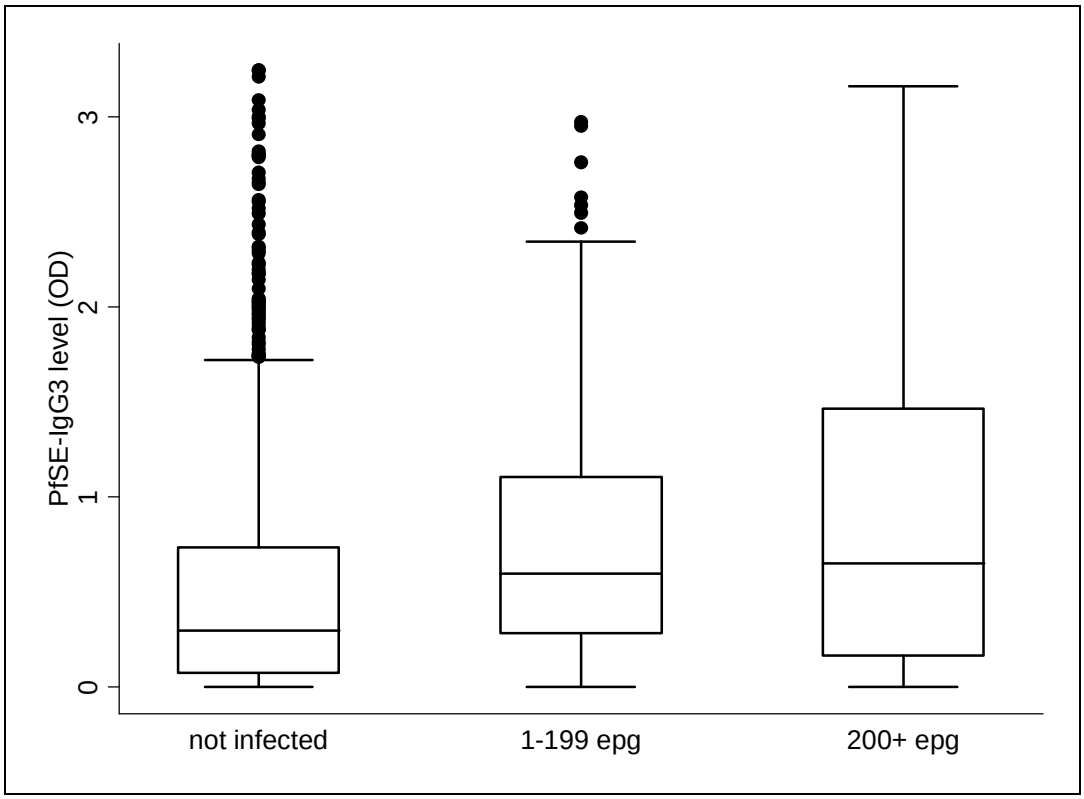
Figure 6.2 Box and whisker plot showing the relationship between PfSE-IgG3 levels and *S. haematobium* infection intensity categorised as not infected (n = 1208), light infection (n = 205) and heavy infection (n = 92).



***The thick line inside each box represents the median value. The lower and upper edge of each box indicates the 25th and 75th percentiles, respectively. The lower and upper whiskers represent the lower and upper values (range), respectively, excluding outliers.**

Overall, children heavily infected with *S. haematobium* had significantly higher PfSE-IgG3 levels compared to uninfected children or those with light infections ($F = 10.23$, $p < 0.001$).

Figure 6.3 Box and whisker plot showing the relationship between PfSE-IgG3 levels and hookworm infection intensity categorised as not infected (n = 1270), 1 – 199 epg (n = 196) and 200+ epg (n = 39).



***The thick line inside each box represents the median value. The lower and upper edge of each box indicates the 25th and 75th percentiles, respectively. The lower and upper whiskers represent the lower and upper values (range), respectively, excluding outliers.**

Overall, children with hookworm infection (epg 200+) had significantly higher PfSE-IgG3 levels compared to uninfected children or those with relatively lighter infections (epg 1-199) ($F = 27.56$, $p < 0.001$).

6.3.3. Relationship between PfSE-IgG3 and organomegaly

The seroprevalence of PfSE-IgG3 was significantly associated with splenomegaly and hepatosplenomegaly. The seroprevalence of PfSE-IgG3 was significantly higher in children with splenomegaly compared to children without splenomegaly ($t = 12.78$, $p < 0.001$). Likewise, the seroprevalence of PfSE-IgG3 was significantly higher in children with hepatosplenomegaly compared to children without hepatosplenomegaly ($t = 24.28$, $p < 0.001$). However, there was no significant difference in seroprevalence of PfSE-IgG3 between children with hepatomegaly and those without hepatomegaly ($\chi^2 = 1.49$, $p = 0.221$). To assess the contribution of PfSE-IgG3 on oganomegaly, multivariate logistic regression analysis was performed with splenomegaly and hepatosplenomegaly as the dependant (outcome) variables (coded as negative or positive) and *P. falciparum* infection, *S. mansoni* infection, *S. haematobium* infection, hookworm infection, PfSE-IgG3 levels and the presence of any helminth infection as the independent variables. PfSE-IgG3 was a significant predictor of both splenomegaly (table 6.5) and hepatosplenomegaly (table 6.6). *S. Mansoni* and *S. haematobium* infections were not predictors of both splenomegaly and hepatosplenomegaly ($p > 0.05$).

Table 6.5 Results of multivariate logistic regression analysis showing predictors of splenomegaly with adjusted odds ratios and p-values (n = 1505).

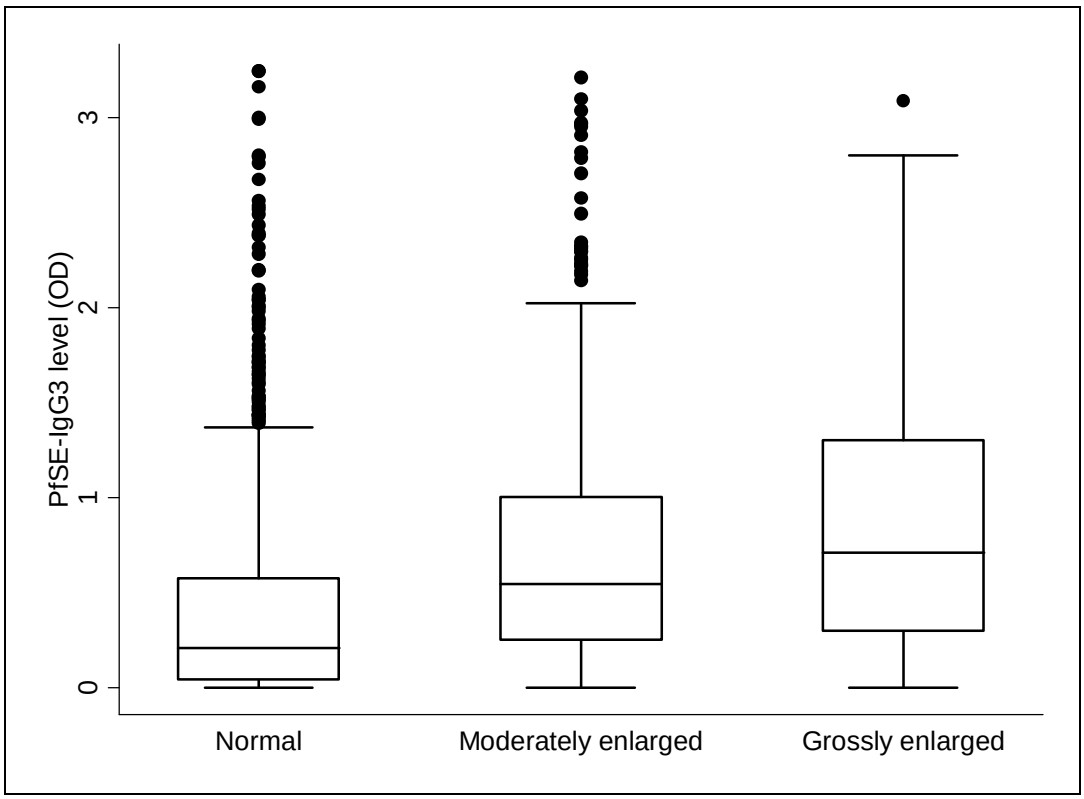
Independent variable	**Adjusted OR (95% CI)**	**P-Value**
Age group	1.696 (1.340 - 2.055)	< 0.001
P. falciparum infection	3.001 (2.367 - 3.823)	< 0.001
Hookworm infection	1.587 (1.178 - 2.138)	< 0.001
PfSE-IgG3	3.885 (2.605 - 5.793)	< 0.001

Table 6.6 Results of multivariate logistic regression analysis showing predictors of hepatosplenomegaly (n = 1505).

Independent variable	**Adjusted OR (95% CI)**	**P-Value**
Age group	1.390 (1.146 - 1.689)	<0.01
P. falciparum infection	2.533 (1.993 – 3.220)	< 0.001
Hookworm	1.641 (1.219 - 2.210)	<0.01
PfSE-IgG3	3.724 (2.399 – 5.780)	< 0.001

Overall, children with detectable PfSE-IgG3 had increased risk of having splenomegaly and hepatosplenomegaly. Older children (> 5 years) and children with blood smear detectable malaria parasitaemia also had increased risk of splenomegaly and hepatosplenomegaly (tables 6.5 and 6.6). Further, PfSE-IgG3 levels were associated with the degree of splenomegaly. Children with grossly enlarged spleens had significantly higher levels of PfSE-IgG3 levels than children with normal spleens or those with moderately enlarged spleens ($F = 83.24$, $p < 0.001$) (figure 6.4).

Figure 6.4 Box and whisker plot showing the relationship between PfSE-IgG3 levels and severity of splenomegaly categorised as normal (n = 911), moderately enlarged (n = 501) and grossly enlarged (n = 93).

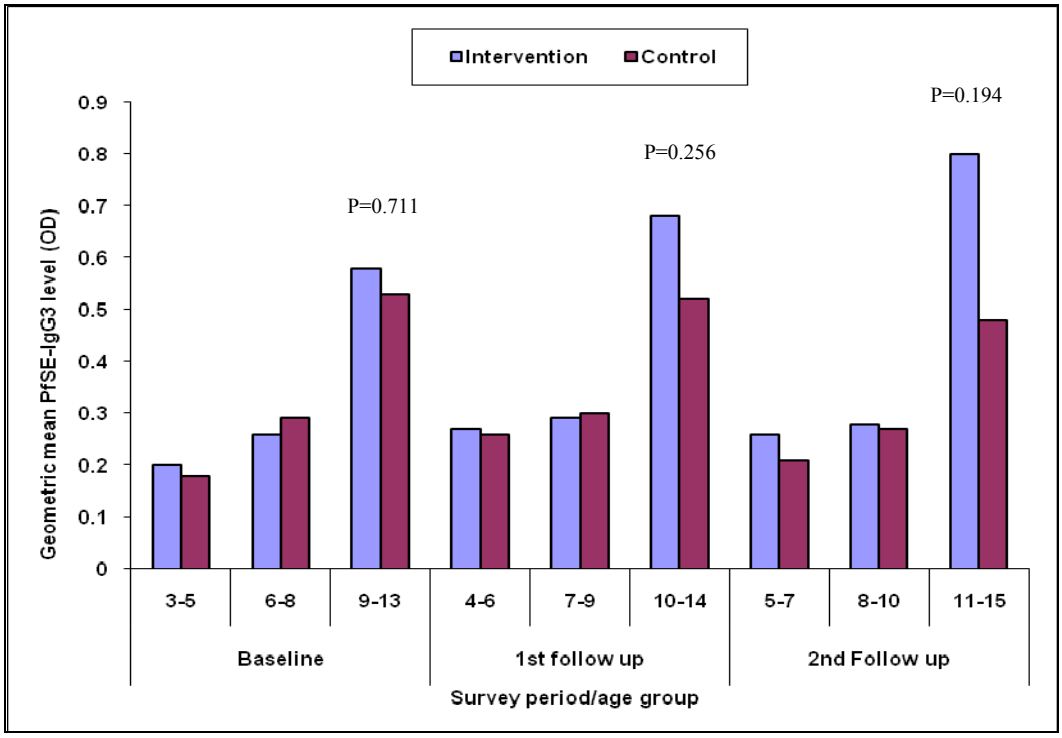


***The thick line inside each box represents the median value. The lower and upper edge of each box indicates the 25th and 75th percentiles, respectively. The lower and upper whiskers represent the lower and upper values (range), respectively, excluding outliers.**

6.3.4. Impact of anthelmintic treatment on PfSE-IgG3 responses

A total of 765 children (see table 5.1 for baseline characteristics) were included in the longitudinal antihelmintic intervention study out of whom 734 children had complete parasitological, ultrasound and PfSE-IgG3 data and were included in the analysis. Out of the 734 children included in the analysis, 629 (85.9%) were seropositive for PfSE-IgG3 without significant differences between the intervention and control groups ($\chi^2 = 0.54$, $p = 0.461$). The seroprevalence of PfSE-IgG3 was 87.7% and 86.7% for the first and second annual follow up surveys, respectively, without significant differences between groups or between baseline and annual follow up surveys ($p > 0.05$). Overall geometric mean PfSE-IgG3 level was 0.264 (95% CI 0.244-0.287) without significant differences between the intervention and control groups ($t = 0.20$, $p = 0.842$). Figure 6.5 shows a comparison of mean PfSE-IgG3 levels over the two years of the intervention stratified by age and randomization groups.

Figure 6.5. Comparison of geometric mean PfSE-IgG3 levels at baseline (n = 734), first follow up (n = 632) and second follow up (n = 564) stratified by age group (in years) and randomization groups.



At baseline, geometric mean PfSE-IgG3 levels increased gradually with age with significantly higher levels in older children (9-13 years) compared to younger children (< 9 years) ($t = 4.56$, $p<0.001$) but without significant differences between the intervention and control groups ($p > 0.05$). During the first annual follow up survey, geometric mean PfSE-IgG3 levels increased significantly for older children (10-14 years) ($t = 4.97$, $p<0.0010$) compared to younger children (< 10 years) where PfSE-IgG3 levels were comparable to baseline levels ($t = 0.72$, $p = 0.473$). The increase in geometric mean PfSE-IgG3 levels was higher for children in the intervention group compared to the control group, though the difference did not reach statistical significance ($t = 1.14$, $p = 0.256$). During the second annual follow up survey, geometric mean PfSE-IgG3 levels for older children (11-15 years) remained significantly higher compared to younger children (<11 years) ($t = 4.24$, $p<0.001$) particularly for the intervention group. Further, geometric mean PfSE-IgG3 levels for younger children (<11 years) remained at baseline levels. For the control group, geometric mean PfSE-IgG3 levels for older children dropped to pre-treatment levels. Overall, a significant increase ($t = 2.23$, $p = 0.027$) in geometric mean PfSE-IgG3 levels was observed following treatment particularly for older children (9-13 years) but without significant differences between groups ($t = 1.31$, $p = 0.194$) (Figure 6.5 and figure 6.6).

Figure 6.6 Comparison of geometric mean PfSE-IgG3 levels for older children (baseline age 9-13 years) for baseline survey (n = 225), first annual follow up survey (n = 126) and second annual follow up survey (n = 114) stratified by treatment group.

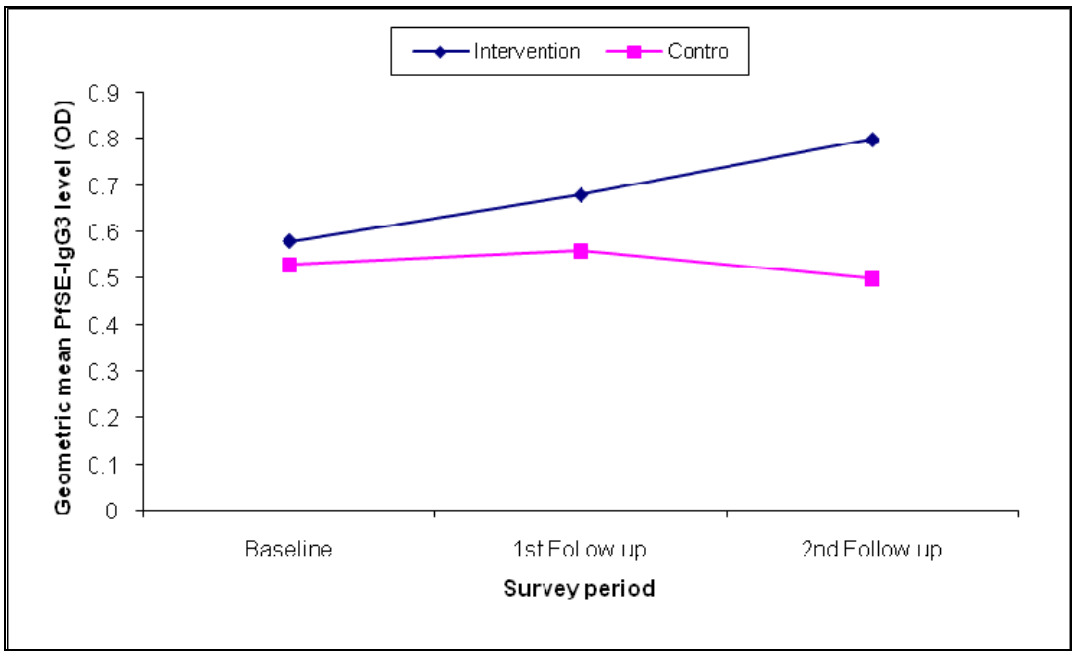


Figure 6.6 shows that overall compared to baseline levels, a significant increase ($t = 2.23$, $p = 0.027$) in geometric mean PfSE-IgG3 levels was observed at the end of the intervention for children in the intervention group. However, there was no significant increase ($t = -0.01$, $p = 0.993$) in geometric mean PfSE-IgG3 levels for children in the control group.

6.4. Discussion

Malaria and helminth co-infections are frequently observed in Sub-Saharan Africa. As helminth infections are associated with Th2 skewed immune responses, they are thought to affect immune responses and clinical outcome of other co-infecting parasites such as malaria parasites. In this study, the relationship between helminth infections (schistosomiasis and hookworm) and immune responses against *P. falciparum* infections (PfSE-IgG3) and the impact of antihelmintic treatment on anti-*P. falciparum* immune responses were examined in school and pre-school children in Magu district, Northwest Tanzania an area co-endemic for both malaria and helminth infections. Baseline seroprevalence and levels of PfSE-IgG3 increased with age and were significantly higher in *P. falciparum*, *S. haematobium* and hookworm infected children compared to un-infected children indicating a possible influence of these infections on immune responses to *P. falciparum* infection. The observed age distribution in seroprevalence and PfSE-IgG3 levels is in accordance with previous reports (Naus *et al*, 2003; Hartgers and Yazdanbackhsh, 2006; Wilson *et al*, 2007a) and reflects the age dependant development of acquired immunity to *P. falciparum* infection which develops slowly over time depending on level of exposure to *P. falciparum* infection. For this reason, immune responses against *P. falciparum* infection are normally lower in young children (4 years or less) than older children (5 years and above) (Franks *et al*, 2001; Taylor *et al*, 1995; Tongren *et al*, 2006). Further, in line with findings from other studies (Booth *et al*, 2004), the seroprevalence and levels of PfSE-IgG3 differed significantly among schools indicating

microgeographical variations in exposure to *P. falciparum* infection. *S. mansoni* infection was not associated with the seroprevalence and levels of PfSE-IgG3 probably due to the fact that majority (about 90%) of *S. mansoni* infections were light to moderate. It has been observed that the association between *P. falciparum* and helminth infections is intensity dependant (Briand *et al*, 2005; Sokhna *et al*, 2004; Waknine-Grinberg *et al*, 2010). As expected, the seroprevalence and PfSE-IgG3 levels correlated with blood smear detected *P. falciparum* infection and intensity. However, the baseline seroprevalence of PfSE-IgG3 was higher compared to the baseline prevalence of *P. falciparum* infection due the fact that the seroprevalence of PfSE-IgG3 is an indicator of cumulative malaria exposure overtime in an area whereas the prevalence of blood smear detectable *P. falciparum* infection indicates only current infections (Drakeley *et al*, 2005). Further, the baseline seroprevalence of PfSE-IgG3 was 82.9% (range 71.8% - 95.9%) significantly higher than what has been reported by other studies in the area (Drakeley *et al*, 2005; Bousema *et al*, 2010) indicating that the study area experiences stable malaria transmission. As has been observed by previous studies (Booth *et al*, 2004b; Wilson *et al*, 2007b), the seroprevalence and levels of PfSE-IgG3 correlated with prevalence and severity of splenomegaly and hepatosplenomegaly indicating that exposure to *P. falciparum* infection plays a role in the development of both splenomegaly and hepatosplenomegaly. This study further observed that children co-infected with *P. falciparum* and *S. haematobium* and those co-infected with *P. falciparum* and hookworm produced significantly higher levels of PfSE-IgG3 responses compared to uninfected children or children with *P. falciparum* only. Increased levels of PfSE-IgG3 in *S. haematobium* and hookworm co-infected children may result from cross-reactivity between antigens of the parasites involved (Mutapi *et al*, 2003; Naus *et al*, 2003; Mwatha *et al*, 2003) or from other synergistic interactions in immune responses of *P. falciparum* and those of co-infecting parasites (Diallo *et al*, 2004; Remoue *et al*, 200; Mwatha *et al*, 2003). A recent study in Zimbabwe (Mutapi *et al* 2003) reported positive association between immune responses against *S. haematobium* and *P. falciparum* infections whereby levels of IgG2, IgG3 and IgG4 directed against schistosome soluble egg antigen (SEA) were significantly associated with levels of IgG2, IgG3 and IgG4 directed against malaria total extract antigens and this observation was attributed to cross reactivity of antigens from the two parasites. Another study in Kenya, Uganda and the Sudan (Naus *et al*, 2003) reported cross-reactivity of IgG3 directed against *P. falciparum* MSP1 and *S. mansoni* adult worm antigens in people exposed to both parasites and attributed this to possession by the two parasites of cross-reactive molecules such as lectins rather than immunological cross-regulation or specific regulatory mechanisms induced by either parasite. It follows therefore that if schistosome infections can cause cross-reactive IgG3 responses, then schistosome co-infections may boost anti-malarial IgG3 responses (Naus *et al*, 2003; Helmby, 2007). On the other hand, the role of helminth co-infections in increased production of PfSE-IgG3 (Th1 immunne response) may be explained in terms of synergistic interactions between anti-*P. falciparum* immune responses and anti-schistosome or other helminth immune responses probably mediated through helminth induced immunomodulation. Although helminth infections are known to be associated with Th2 immune responses, the direction of immunomodulation is determined by the balance between Th1 and Th2 responses depending on the stage and timing of infections, the species and strains of parasites involved, the intensity of infection and more importantly the age and background immunity of the host (Hartgers *et al*, 2006). Acute schistosome infection for example may enhance production of Th1 cytokines (hence higher levels of IgG1 and IgG3 antibody responses) (Hartgers *et al*, 2006). Therefore, depending on factors listed above, a Th1 response may still predominate over a Th2 response in some cases of malaria-helminth co-infections (Helmby *et al*, 1998; Hartgers *et al*, 2006; Diallo *et al*, 2004). It is also worth mentioning that *S. haematobium* infection has been reported to protect against malaria infection (Briand *et al*, 2005; Lyke *et al*, 2005) through mechanisms not yet clearly explained. The results of the current study also suggest that the presence of concurrent helminth infections (*S. haematobium* and hookworm) caused increased production of PfSE-IgG3 probably through cross reactive antigens or other mechanisms such as

immune modulation. This explanation is supported by parasitological findings (Figure 3.3, figure 3.4, table 3.5) which showed negative correlation between malaria parasite density and helminth egg counts indicating that presence of helminth infection (*S. mansoni* and *S. haematobium*) suppressed multiplication of malaria parasites probably through cross-reactivity between anti-schistosome and anti-malarial specific immune responses as observed by previous studies (Naus *et al*, 2003; Helmby, 2007; Mutapi *et al*, 2007). In line with reports from other studies (Mwatha *et al*, 2003; Wilson *et al*, 2009), PfSE-IgG3 levels were positively associated with prevalence and severity of splenomegaly and hepatosplenomegaly indicating involvement of chronic exposure to malaria as the causative factor for development of both. The study of Wilson *et al* (2009) in Kenya demonstrated a similar relationship between *S. mansoni* infection, anti-*P. falciparum* IgG3 (Pfs-IgG3) (as a marker of chronic exposure to malaria) and hepatosplenomegaly whereby Pfs-IgG3 levels correlated with a pro-inflammatory immune response characterised by higher production of IL-12p70 which in turn correlated with hepatosplenomegaly. Contrary to findings of studies in Kenya (Fulford *et al*, 1991; Mwatha *et al* 2003; Wilson *et al*, 2007b) where PfSE-IgG3 was shown to be associated with hepatomegaly, in the current study PfSE-IgG3 was not associated with hepatomegaly indicating that in the studied population, chronic exposure to malaria infection played an insignificant role in the aetiology of hepatomegaly compared to *S. mansoni* infection (see table 4.6). A study in Zimbabwe (Whittle *et al*, 1969) demonstrated age profiles in the aetiology of hepatomegaly. While malaria infection was significantly associated with hepatomegaly in young children (0-4 years), *S. mansoni* infection was the significant cause of hepatomegaly in children aged 5 – 9 years. However, it is generally known that both malaria and *S. mansoni* infections cause hepatosplenomegaly (including hepatomegaly) in areas where these diseases are co-endemic (Fulford et al, 1991; Whittle *et al*, 1969; Gryseels *et al*, 1987; Sowunmi *et al*, 1996; Sowunmi *et al* 2001; Booth *et al*, 2004; Wilson *et al*, 2007b). Thus chronic exposure to malaria and *S. mansoni* infections may cause hepatosplenomegaly through immune mediated inflammatory processes asscociated with secretion of pro-inflammatory cytokines (Diallo *et al*, 2004; Remoue *et al*, 2003). .

This study also observed that antihemintic treatment using the drugs praziquantel against schistosomiasis and albendazole against STH lead to overall increase in PfSE-IgG3 levels particularly for older children (baseline age group 9-13 years). An explanation for the observed increase in PfSE-IgG3 levels could be the effect of the antihelmintic treatment using praziquantel on anti-schistosome immune responses. Previous studied have demonstrated that treating schistosome infections using praziquantel exposes higher quantities of schistosome antigens from damaged tegument of dying schistosome parasites to the immune system (Grogan *et al*, 1996; Satti *et al*, 1998; Mutapi *et al*, 1998; Mutapi *et al*, 2005) which results in increased immune responses in terms of specific antibody subclasses and levels produced. In this case for example, praziquantel treatment may lead to increased production of anti-schistosome IgG1 and IgG3 subclasses which in turn may interact with anti-*P. falciparum* immune responses to cause increased production of PfSE-IgG3 through mechanisms previously described (Remoue *et al* 2003; Diallo *et al*, 2004; Mutapi *et al*, 2003). This explanation is supported by the fact that all children randomized to the intervention and control groups were initially treated for schistisomiasis using praziquantel immediately after the baseline survey. The fact that the increase in PfSE-IgG3 levels following treatment was observed particularly in older children (baseline age group 9-15 years) reflects the age groups most at risk of *P. falciparum* and helminth (schistosome and hookworm) co-infections in which interaction between these infections is most likely to occur (Brooker *et al*, 2006; Brooker *et al*, 2007; Mwangi *et al*, 2006). An alternative explanation for the observed increase in PfSE-IgG3 levels could be changes in the dynamics of malaria transmission in the studied population. The prevalence of malaria increased significantly from baseline levels of 29.8% to 44.9% and 42.4% for the intervention and control groups, respectively, during the first annual follow up survey. The increase in overall prevalence of *P. falciparum* infection might have caused the observed increase in PfSE-

IgG3 levels observed during the first annual follow up survey. This explanation is in line with observations made in Zimbabwe (Reilley *et al*, 2008) whereby an increase in anti-*P. falciparum* IgG3 levels was observed 6 weeks after treatment of *S. haematobium* infection using praziquantel and was attributed to changes in dynamics of *P. falciparum* infections not antihelmintic treatment. Yet another explanation for increased production of PfSE-IgG3 levels could be that there were improved anti-*P.falciparum* immune responses following treatment of helminth infections. This means that treatment of helminth infections lead to the predominance of Th1 immune responses (anti-*P. falciparum*) characterised by increased production of Th1 cytokines and hence increased production of anti-*P. falciparum* IgG1 and IgG3. However, this would have been reflected by decreased prevalence of malaria infections (prevalence and infection intensity) during the first annual follow up survey which was not the case. However, the observed increase in PfSE-IgG3 levels could be as a result of a confounding effect of increased in age as children age increase by one and two years at first and second follow up examinations, respectively compared to baseline age. One question might be on the duration of the effect resulting from antihelmintic treatment or the effect of changes in malaria transmission dynamics on anti-*P. falciparum* immune responses. A study in Zimbabwe (Mutapi *et al*, 1998) showed that antigens released from disintegration of schistosome worms following treatment declined after 36 weeks (about 8 months) resulting into a corresponding decline in anti-schisosome antibodies produced. Further, a recent study in Kenya (Kinyanjui *et al*, 2007) found that anti-*P. falciparum* IgG1 and IgG3 responses against recombinant *P. falciparum* merozite antigens have a short life span and levels declined within 6 weeks unless boosted by further *P. falciparum* infections. The observations made by the two studies may explain the apparent drop in PfSE-IgG3 levels observed during the second annual follow up particularly for the control group. However, the current study was conducted in an area with stable malaria transmission and hence children were exposed to continuous malaria parasite challenge throughout the study period. It is possible therefore that an initial boost in PfSE-IgG3 production may have been indirectly caused by treatment of schistosomiasis using praziquantel and then maintained through subsequent challenge by malaria parasites. Therefore one or more mechanisms may have been involved in the observed increase in PfSE-IgG3 levels. Another question would be whether the observed increase in PfSE-IgG3 levels was reflected in protection from subsequent *P. falciparum* infections and disease. There was an overall reduction in malaria prevalence in the study population at the end of the antihelmintic intervention (Table 5.2) and it is possible that the observed increase in anti-*P. falciparum* immune responses contributed to this. However, this needs to be confirmed by further studies. In conclusion, this study has demonstrated that *P. falciparum* and *S. haematobium* co-infection and *P. falciparum* and hookworm co-infections are positively correlated with PfSE-IgG3 levels indicating positive interactions of *S. haematobium* and hookworm infections on anti-*P. falciparum* immune responses. Further, treatment of schistosomiasis using praziquantel and STH (hookworm) infections using albendazole caused a significant increase in PfSE-IgG3 levels probably through mechanisms involving altered immune responses to schistosome antigens following treatment. However, it was not clear if this increase was associated with improved protection against *P. falciparum* infection and disease.

6.5. References

Artavanis-Tsakonas, K and Riley, E, M. (2002). Innate immune response to malaria: rapid induction of IFN-gamma from human NK cells by live Plasmodium falciparum-infected erythrocytes. *J Immunol*, **169** (6): 2956-2963.

Bousema, T., Youssef, R. M., Cook, J., Cox, J., Alegsns, V. A., Amran, J., Noor, A. M., Snow, R. W., Drakeley, C. (2010). Serological markers for detecting malaria in areas of low endemicity in Somalia. *Emerg Infect Dis*, **16** (3): 392-399.

Booth, M., Vennervald, B. J., Kenty, L., Butterworth, A. E., Kariuki, H. C., Kadzo, H., Ireri, E., Amaganga, C., Kimani, G., Mwatha, J. K., Otedo, A., Ouma, J. H., Muchiri, E., Dunne, D. W. (2004a). Micro-geographical variation in exposure to Schistosoma mansoni and malaria, and exacerbation of splenomegaly in Kenyan school-aged children. *BMC Infect Dis,* **4**: 13.

Booth, M., Vennervald, B. J., Butterworth, A. E., Kariuki, H. C., Amaganga, C., Kimani, G., Mwatha, J. K., Otedo, A., Ouma, J. H., Dunne, D. W. (2004b). Exposure to malaria affects the regression of hepatosplenomegaly after treatment for Schistosoma mansoni infection in Kenyan children**.** *BMC Med*, **27**: 2: 36.

Briand, V., Watier, L., Le Hesran, J., Garcia, A., Cot, M. (2005). Co-infection with *Plasmodium falciparum* and *Schostosoma haematoboum*: Protective effect of Schostosomiasis on malaria in Senegalese children? *Am. J. Trop. Med. Hyg*, **72:** 702-707.

Brooker, S., Clements, A. C., Hotez, P. J., Hay, S. I., Tatem, A. J., Bundy, D. A., Snow, R. W. (2006). The co-distribution of Plasmodium falciparum and hookworm among African schoolchildren. *Mal J*, **5**: 99.

Brooker, S., Akhwale, W., Pullan, R., Estambale, B., Clarke, S. E., Snow, R. W., Hotez, P. J. (2007). Epidemiology of plasmodium-helminth co-infections in Africa: Population at risk, potential impact on anaemia and prospwts for combined control. *Am. J. Trop. Med. Hyg,* **77**: S88-S98.

Choudhury, H. R., Sheikh, N.A., Bancroft, G. J., Katz, D. R., De, Souza, J. B. (2000). Early nonspecific immune responses and immunity to blood-stage nonlethal Plasmodium yoelii malaria. *Infect Immun*, **68** (11): 6127-32.

Diallo, T. O., Remoue, F., Schacht A, M., Charrier, N., Dompnier, J. P., Pillet, S., Garraud O., N'diaye A. A., Capron, A., Capron M., Riveau, G. (2004). Schistosomiasis co-infection in humans influences inflammatory markers in uncomplicated Plasmodium falciparum malaria. *Par Immunol,* **26** (8-9): 365-369.

Drakeley, C. J., Corran, P. H., Coleman, P. G., Tongren, J. E., McDonald, S. L., Carneiro, I., Malima, R., Lusingu, J., Manjurano, A., Nkya, W. M., Lemnge, M. M., Cox, J., Reyburn, H., Riley, E. M. (2005). Estimating medium- and long-term trends in malaria transmission by using serological markers of malaria exposure. *Proc Natl Acad Sci U S A*, **102** (14): 5108-5113.

Druilhe, P., Tall, A., Sokhna, C. (2005). Worms can worsen malaria. Towards a new means to roll back malaria? *Trends Parasitol*, **21**: 359-362.

Druilhe, P., Sauzet, J. P., Sylla, K., Roussilhorn, C. (2006). Worms can alter T cell responses and induce regulatory T cells to experimental malaria vaccines. *Vaccine,* **24** (23): 4902-4904.

Egwunyenga, O. A., Ajayi, J. A., Nmorsi, O. P. G., Duhlinska-Popova., D. D. (2001). Plasmodium/intestinal helminth co-infections among pregnant Nigerian women. *Mem. Inst. Oswaldo Cruz,* **96** (8): 1055-1059.

Fulford, A. J., Mbugua, G. G., Ouma, J. H., Kariuki, H. C., Sturrock, R. F., Butterworth, A. E. (1991). Differences in the rate of hepatosplenomegaly due to Schistosoma mansoni

infection between two areas in Machakos District, Kenya. *Trans Roy Soc Trop Med Hyg,* **85** (4): 481-488.

Franks, S., Koram, K. A., Wagner, G. E., Tetteh, K., McGuinness, D., Wheeler, J. G., Nkrumah, F., Ranford-Cartwright, L., Riley E. M. (2001). Frequent and persistent, asymptomatic Plasmodium falciparum infections in African infants, characterized by multilocus genotyping. *Infect Dis,* **183** (5): 796-804.

Good, M. F., Xu H., Wykes, M., Engwerda, C. R. (2005). Development and regulation of cell-mediated immune responses to the blood stages of malaria: implications for vaccine research. *Annu Rev Immunol*, **23**: 69-99.

Grogan, J. L., Kremsner, P. G., Van Dam, G. J., Metzger, W., Mordmüller, B., Deelder A. M., Yazdanbakhsh, M. (1996). Antischistosome IgG4 and IgE responses are affected differentially by chemotherapy in children versus adults. *J Infect Dis,* **173** (5): 1242-1247.

Gryseels, B., Polderman, A. M. (1987). The morbidity of schistosomiasis mansoni in Maniema (Zaire). *Trans Roy Soc Trop Med Hyg***, 81** (2): 202-209

Gryzch, J. M., Pearce, E., Cheever, A., Caulada, Z. A., Casper, P., Heiny, S., Lewis, F., Sher, A. (1991). Egg deposition is the major stimulus for the production of Th2 cytokines in Murine schistosomiasis mansoni. *J. Immunol,* **146**: 1322-1327.

Hartgers, F. C and Yazdanbackhsh, M. (2006). Co-infection of helminths and malaria. Modulation of immune responses to malaria *Par Immunol,* **28** (10): 497-506.

Hartgers, F. C., Obeng, B. B., Kruize, Y. C., Dijkhuis, A., McCall M., Sauerwein R.W., Luty A. J., Boakye, D. A., Yazdanbakhsh, M. (2009). Responses to malarial antigens are altered in helminth-infected children. *J. Infect. Dis*, **199** (10): 1528-1535.

Helmby, H.; Kulberg., M., Troye-Blomberg, M. (1998). Altered immune responses in mice with concomitant *Schistosoma mansoni* and *Plasmodium chabaudi* infection. *Infect Iimmun*, **66**: 5167-5174.

Helmby, H. (2007). Schistosomiasis and malaria: Another piece of the cross-reactivity puzzle. *Trends Parasitol*, **23** (3): 88-90.

Kinyanjui, S. M., Conway, D. J., Lanar, D. E., Marsh K. (2007). IgG antibody responses to Plasmodium falciparum merozoite antigens in Kenyan children have a short half-life. *Mal J*, **28**: 6: 82.

Lewinsohn, R. (1975). Anaemia in mice with concomitant *S. Mansoni* and *P. berghei yoelii* infection. *Trans. Roy. Soc. Trop. Med. Hyg,* **69** (1): 51-56.

Lyke., K. E., Dabo, A., Sangare, L., Arama, C., Daou, M., Diarra, I., Plowe, C. V., Doumbo, O. K., Sztein, M. B. (2006). Effect of concormittant *Schistosoma haematobium* infection on the serum cytokine levels elicited by acute Plasmodium falciparum malaria infection in Malian children. *Infect Immun*, **74** (10): 5718-5724.

Maizels, R. M., Bundy, D. A., Serkirk, M. E., Smith, D. F., Anderson, R. M. (1993). Immunological modulation and evarsion by helminth parasites in human populations. *Nature*, **365**: 797-805.

Maizels, R. M., Balic, A., Gomez-Escobar, N., Nair, M., Taylor, M. D., Allen, J. E. (2004). Helminth parasites–masters of regulation. *Immunol Rev* **201:** 89–116.

Mohan, K., Moulin, P., Stevenson, M. M. (1997). Natural killer cell cytokine production, not cytotoxicity, contributes to resistance against blood-stage Plasmodium chabaudi AS infection. *J Immunol*, **159** (10): 4990-4998.

Mosmann, T. R and Coffman, R. L. (1989). TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Ann Rev Immunol*, **7**: 145–173.

Mutapi, F., Ndhlovu, P. D., Hagan, P., Spicer, J. T., Mduluza, T., Turner, C. M., Chandiwana, S. K., Woolhouse, M. E. (1998). Chemotherapy accelerates the development of acquired immune responses to Schistosoma haematobium infection. *J Infect Dis*, **178** (1): 289-293.

Mutapi, F., Hagan, P., Woolhouse, M. E., Mduluza, T., Ndhlovu, P. D. (2003). Chemotherapy-induced, age-related changes in antischistosome antibody responses. *Par Immunol*, **25** (2): 87-97.

Mutapi, F., Burchmore, R., Mduluza, T., Foucher, A., Harcus, Y., Nicoll, G., Midzi, N., Turner, M., Maizels, R. M. (2005). Praziquantel treatment of individuals exposed to Schistosoma heamatobium enhances serological recognition of defined parasite antigens. *J. Infect. Dis*, **192**: 1108-1118.

Mutapi, F., Roussilhon, C., Mduluza, T., Druilhe, P. (2007). Anti-malaria humoral responses in children exposed to Plasmodium falciparum and Schistosoma haematobium. *Mem Inst Oswaldo Cruz*, **102** (3): 405-409.

Mwangi, T. W., Bethony, J. M., Brooker, S. (2006). Malaria and helminth interactions in humans: An epidemiological viewpoint. *An. Trop. Med. Parasitol*, **100** (7): 551-570.

Mwatha, J. K., Jones F. M., Mohamed, G., Naus, C. W., Riley, E. M., Butterworth, A. E., Kimani, G., Kariuki, C. H., Ouma, J. H., Koech, D., Dunne, D. W. (2003). Associations between anti-Schistosoma mansoni and anti-Plasmodium falciparum antibody responses and hepatosplenomegaly in Kenyan schoolchildren.*J Infect Dis*. **187** (8): 1337-1341.

Nacher, M., Singhasivanon, P., Yimsamran, S., Manibunyong, W., Thanyavanich, N., Wuthisen, P, Looareesuwan, S. (2002). Intestinal helminth infections are associated with increased incidence of *Plasmodium falciparum* malaria in Thailand. *Journal of Parasitology*, **88** (1): 55-58.

Naus, C. W., Jones, F. M., Satti, M. Z., Joseph, S., Riley, E. M., Kimani, G., Mwatha, J. K., Kariuki, C. H., Ouma, J. H., Kabatereine, N. B., Vennervald, B. J., Dunne D. W. (2003). Serological responses among individuals in areas where both schistosomiasis and malaria are endemic: cross-reactivity between Schistosoma mansoni and Plasmodium falciparum. *J Infect Dis,* **187** (8): 1272-82.

Reilly, L., Magkrioti, C., Mduluza, T., Cavanagh, D. R., Mutapi, F. (2008). Effect of treating Schistosoma haematobium infection on Plasmodium falciparum-specific antibody responses. *BMC Infect Dis*, **8:** 158.

Remoue, F., Diallo, T. O., Angeli, V., Hervé, M., De Clercq D., Schacht, A. M., Charrier, N., Capron, M., Vercruysse, J., Ly, A., Capron, A., Riveau, G. (2003). Malaria co-infection in children influences antibody response to schistosome antigens and inflammatory markers associated with morbidity. *Trans. R. Soc. Trop. Med. Hyg,* **97** (3): 361-364.

Satti, M. Z., Lind, P., Vennervald, B. J., Sulaiman, S. M., Daffalla, A. A., Ghalib, H. W. (1996). Specific immunoglobulin measurements related to exposure and resistance to Schistosoma mansoni infection in Sudanese canal cleaners. *Clin Exp Immunol*, **106** (1): 45-54.

Sokhna, C., Le Hesran, J. Y., Mbaye, P. A., Akiana, J., Camara, P., Diop, M., Ly, A., Druilhe, P. (2004). Increased malaria attacks among children presenting concomitant infections by *S. mamsoni* in Senegal. *Mal Jl*, **3:** 43.

Sowunmi, A. (1996). Hepatomegaly in acute falciparum malaria in children. *Trans R Soc Trop Med Hyg*, **90** (5): 540-542.

Sowunmi, A., Adedeji, A. A., Sowunmi, C. O., Falade, C. O., Falade, A. G., Ohaeri, B., Happi, T. C., Oduola, A. M. (2001). Clinical characteristics and disposition kinetics of the hepatomegaly associated with acute, uncomplicated, Plasmodium falciparum malaria in children. *Ann Trop Med Parasitol*, **95** (1): 7-18.

Spiegel, A., Tall, A., Raphenon, G., Trape, J. F., Druilhe, P. (2003). Increased frequency of malaria attacks in subjects co-infected by intestinal worms and *Plasmodium falciparum* malaria. *Trans. Roy. Soc. Trop. Med. Hyg*, **97:** 198-199.

Taylor, R. R., Smith, D. B., Robinson, V. J., McBride, J. S., Riley, E. M. (1995). Human antibody response to Plasmodium falciparum merozoite surface protein 2 is serogroup specific and predominantly of the immunoglobulin G3 subclass. *Infect Immun*, **63** (11): 4382-4388.

Tongren, J. E., Drakeley, C. J., McDonald, S. L., Reyburn, H. G., Manjurano, A., Nkya, W. M., Lemnge, M. M., Gowda, C. D., Todd, J. E., Corran, P. H., Riley, E. M. (2006). Target antigen, age, and duration of antigen exposure independently regulate immunoglobulin G subclass switching in malaria. *Infect Immun*, **74** (1): 257-264.

Tschikuka., J. G., Scott, M. E., Gray-Donald, K., Kalumba, O. N. (1996). Multiple infection with plasmodium and helminths in communities of low and relatively high socio-economic status. *Ann Trop Med Parasitol*, **90** (3): 277-293.

Waknine-Grinberg, J. H., Gold, D., Ohayon, A., Flescher, E., Heyfets, A., Doenhoff, M. J., Schramm, G., Haas, H., Golenser, J. (2010). *Schistosoma mansoni* infection reduces the incidence of murine cerebral Malaria. *Mal J,* **9**: 1186.

Wilson S., Booth, M., Jones, F. M., Mwatha, J. K., Kimani, G., Kariuki, H. C., Vennervald, B. J., Ouma J. H., Muchiri, E., Dunne, D. W. (2007a). Age-adjusted Plasmodium falciparum antibody levels in school-aged children are a stable marker of microgeographical variations in exposure to Plasmodium infection. *BMC Infect Dis*, **29:** 7: 67.

Wilson, S., Vennervald B. J., Kadzo, H., Ireri, E., Amaganga, C., Booth, M., Kariuki, H. C., Mwatha, J. K., Kimani, G., Ouma, J. H., Muchiri, E., Dunne, D. W. (2007b). Hepatosplenomegaly in Kenyan schoolchildren: exacerbation by concurrent chronic exposure to malaria and Schistosoma mansoni infection. *Trop Med Int Health*, **12** (12): 1442-1449.

Whittle, S., Gelfand, M., Sampson, E., Purvis, A., Weber, M. (1969). Enlarged livers and spleens in an area endemic for malaria and schistosomiasis. *Trans. Roy. Soc. Trop. Med. Hyg*, **63**: 353-361.

Chapter 7: General discussion and conclusions

Multiple parasite infections involving *P. falciparum* and helminths are common in Sub-Saharan Africa and interactions that affect disease severity and outcome would be expected to occur. Individuals who harbour multiple parasite infections have increased risk of severe disease and mortality. The overall objective of the current study was to contribute to the knowledge on malaria and helminth (schistosomes and STH) co-infections in school and pre-school children with a focus on the epidemiology of these infections, assessment of morbidity related to these infections and examining possible interactions among these infections. The study also examined the effect of schistosome and STH infections on *P. falciparum* specific antibody responses and the impact of an anthelmintic intervention on malaria infection, *P. falciparum* specific antibody responses, prevalence and intensity of schistosome and STH infections, haemoglobin concentrations and anaemia. The ultimate goal was to provide evidence based information that will support informed policy making on integrated parasitic diseases control programmes among school and pre-school children in Tanzania and elsewhere.

The study has provided evidence on the significance of malaria, schistosoiasis and hookworm as major public health problems in school and pre-school children in the study area not only as single infections but also as concurrent infections. High prevalence rates and intensity of *S. mansoni*, *P. falciparum*, *S. haematobium* and hookworm infections were found in the studied population, often associated with low haemogloin levels and anaemia pointing out to the disease burden which these infections can cause to affected populations. Although variations existed among schools, age groups and sexes with regard to prevalence and intensity as well as associated morbidity, all schools, sexes and age groups were affected. Multiple parasite infections involving two or more parasites were frequently encountered the majority of which involved *P. falciparum*, *S. mansoni* and *S. haematobium*. It was thus not surprising that majority of morbidity such as anaemia and organ pathology was associated with prevalence and intensity of these infections which in turn shows the importance of targeting disease control interventions particularly to these three parasites. Hookworm infections were not observed with high infection rates which was expected as the prevalence curves for this helminth specie peaks in adulthood. However, attention should be given to this parasite when planning for disease control interventions in the adult population.

The study has demonstrated associations involving all four parasite species investigated. The most notable synergistic associations observed were between *P. falciparum* and *S. Haematobium* and low Hb levels and anaemia; *P. falciparum* and *S. mansoni* were associated with hepatomegaly, *P. falciparum* and hookworm with splenomegaly and hepatosplenomegaly and *S. haematobium* and hookworm with urinary tract pathology. Further, positive associations were observed including prevalence of malaria parasitaemia and hookworm infection; malaria parasite density and hookworm infection and prevalence and intensity of both *S. mansoni* and *S. haematobium* infections and hookworm infection. Negative associations were observed between malaria parasite density and both *S. haematobium* and *S. mansoni* infections. Combined, these observations suggest that in multiple concurrent parasite infections, both positive and negative associations may occur depending on the parasite species involved, the stage and timing of infections, the intensity of infection and probably host factors such as age and immune responses. It may further be stated that observed positive association such as *P. falciparum* and *S. haematobium* with anaemia support the hypothesis that concurrently infected individuals are at the highest risk of severe disease and morbidity. This in turn points to the importance of considering an integrated approach when planning disease control programmes in areas where multiple parasite infections occur. Both clinical and sonographic examinations revealed *S. mansoni, S. haematobium* and *P. falciparum* related morbidity such as

PPF, hepatomegaly, splenomegaly and urinary bladder pathology in children as young as 5 years which correlated with prevalence and infection intensity of all three parasite infections. In addition to providing evidence on the endemicity of *P. falciparum, S. mansoni, S. haematobium* and hookworm infections in the studied communities, these findings also provide an insight into the type and magnitude of morbidity which can be caused by these infections particularly in school and pre-school children. The public health implications of the findings is that an integrated approach should be considered when planning for schistosomiasis and STH control interventions in school and pre-school children. Further, children above the age of 5 years should also be considered when designing malaria control interventions contrary to the current practice whereby the focus is on children under the age of five years and pregnant women. The observation of higher prevalence of *S. mansoni and S. haematobium* related pathology (chapter 4) in children as young as 5 years suggests that use of ultrasound as a tool for morbidity assessment and evaluation of the impact of antihelmintic treatment should be included as part of surveillance and control activities for schistosomiasis in school and pre-school children.

In line with previous studies, this study demonstrated the impact of antihelmintic treatment using praziquantel and albendazole on the prevalence and infection intensity of *S. mansoni, S. haematobium* and hookworms as well as on observed frequency of multiple parasite infections. The intervention also led to improvement in Hb levels and successfully reversed schistosomiasis related morbidity particularly PPF, hepatosplenomegaly and urinary bladder pathology. Whereas there was about 100% resolution of urinary tract morbidity caused by *S. haematobium* infection one year after anthelmintic treatment, some *S. mansoni* related morbidity persisted after the two years of anthelmintic treatment. The interpretation of these results is that praziquantel and albendazole are still reliable drugs for the treatment of schistosomiasis and STH, the efficacy of praziquantel being higher for *S. haematobium* compared to *S. mansoni*. Large scale mass drug administration using these drugs could potentially result in public health impact in terms of reductions of prevalence, infection intensity and morbidity of single and multiple parasitic infections. However, the impact of the antihelmintic intervention was limited in terms of reductions in prevalence and intensity of *S. mansoni* infection. A similar finding was observed for morbidity indicators such as hepatomegaly and splenomegaly which could be attributed to both *S. mansoni* and *P. falciparum*. These observations suggest that repeated treatments in a year over several years may be necessary in areas with higher transmission of *S. mansoni*. Further, treatment for *P. falciparum* in addition to treatment for schistosomiasis may be necessary to completely reverse *S. mansoni* related pathology in areas endemic for both *S. mansoni* and *P. falciparum* infections. On the other hand, the antihelmintic treatment did not have an impact on malaria infection (prevalence, malaria parasite density and frequency of malaria attacks) suggesting that although helminth infections may have an effect on malaria infection in an intensity dependent manner as was demonstrated in the baseline study, other factors such as vector dynamics and seasonal variations in temperature and rainfall may still be important in determining overall malaria transmission in an area. This aspect however needs further investigations.

This study has also demonstrated that *S. haematobium* and hookworm infections influences anti-*P. falciparum* specific immune responses. Baseline seroprevalence and levels of PfSE-IgG3 correlated positively with the prevalence and intensity of *P. falciparum, S. haematobium* and hookworm infections. Further, children co-infected with *P. falciparum* and *S. haematobium* and those co-infected with *P. falciparum* and hookworm produced significantly higher levels of PfSE-IgG3 responses compared to uninfected children or children infected with *P. falciparum* only. Furthermore, findings of the longitudinal follow up study showed that treatment of schistosomiasis and hookworm infections using praziquantel and albendazole was followed by significant increase in PfSE-IgG3 levels. These observations support the idea that *S. haematobium* and hookworm

infections and anthelmintic treatment influences *anti-P.falciparum* immune responses particularly in children aged 9 years and above. Interestingly, in addition to the observed association between PfSE-IgG3 levels and hookworm infection, parasitological data (Table 3.4) also showed a significant positive association between prevalence of malaria parasitaemia and hookworm infection. These two observations which are supported by findings of Hillier *et al* (2008) in Uganda provides evidence supporting the existence of a true biological association between *P. falciparum* and hookworm infections which is immunologically mediated.

An important question remains on the protective role of the observed increase in PfSE-IgG3 levels due to *S. haematobium* and hookworm infections and antihelmintic treatment against malaria infection and disease. Supposedly the observed interactions would exert an influence on development of acquired immunity against *P. falciparum* malaria and on how co-infected individuals mount an immune response against subsequent *P. falciparum* infections and disease. Overall, the findings of the current study suggest that helminth co-infections could boost immune responses against *P. falciparum* infections and enhance protection from malaria in co-infected individuals, a view which contrasts findings of previous studies which reported increased frequency of malaria attacks in co-infected individuals (Nacher *et al*, 2002; Spiegel *et al*, 2003; Sokhna *et al*, 2004). Indeed parasitological findings (see table 5.2) showed that at the end of the intervention, malaria parasite prevalence decreased in both the intervention and control group to significant lower levels compared to baseline and 1st follow up levels. However it was not clear whether this reduction in malaria transmission resulted from the antihelmintic intervention or from seasonal variations in malaria transmission. On the other hand, there is evidence suggesting that increased production of PfSE-IgG3 could affect the Th1/Th2 balance towards an immune reaction favouring development of schistosomiasis pathology by increasing levels of inflammatory markers associated with development of schistosomiasis morbidity such as IFN-γ, TNF-RII and higher levels of IL-10 (Mwatha *et al*, 1998; Ouma *et al*, 2001; Falloon *et al*, 2000; Montenegro *et al*, 1999). Thus the influence of helmith co-infections on anti-*P. falciparum* immune responses could have a protective effect on malaria infection and disease but also a harmfull effect by increasing production of inflammatory markers associated with schistosomiasis morbidity. This aspect of the study however needs further investigation.

In conclusion the main findings of the current study may be summarized as follows:

- Malaria and helminth infections are very common in school and pre-school children in Magu district and are associated with varying degrees of morbidity which correlate well with both prevalence and intensity of the infections.

- Concurrent heminth infections exacerbates malaria related morbidity such as anaemia, splenomegaly and hepatosplenomegaly in co-infected individuals. Further, helminth infections (*S. mansoni* and *S. haematobium*) are negatively correlated with malaria parasite density in co-infectied individuals. These observations provide evidence of the importance of malaria and helmihtn co-infections and of the synergistic interactions which occur in co-infected individuals. The public health importance of anaemia in school children and pregnant women particularly in Sub-Saharan Africa is known and parasitic infections including malaria and helminths are known to be main contributors. It is evident therefore that their combined presence in the same individuals may result into increased risk of anaemia.
- Helminth infections (*S. haematobium* and hookworm infections) and/or treatment of schistosome infections alter immune responses directed against *P. falciparum* infection. This observation points out to possible involvement of immunological mechanisms as the

underlying cause of the observed interactions between helminth and *P. falciparum* infections.

- The anthelmitic intervention had limited impact on malaria infection; however the intervention significantly reduced prevalence and infection intensity of schistosome and hookworm infection and the prevalence of multiple parasitic infections. Further, the intervention caused a significant improvement in Hb levels and hence reduction in the prevalence of anaemia. This observation is a clear demonstration that an integrated approach to the control of parasitic diseases could be more effective in relieving overall disease burden resulting from parasitic co-infections. The impact on disease burden could be further improved particularly in school and pre-school aged children by combining helminth control and malaria such as anthelmintic treatment and malaria chemoprophylaxis, respectively.

Future studies are needed to address knowledge gaps in the understanding of malaria and helminth co-infections in human populations. Specifically future studies should focus on:

- The effect of hookworm infection on malaria and other helminth infections: The current study demonstrated significant associations between hookworm and *P. falciparum* infections (prevalence and infection intensity). Likewise, the current study observed positive associations between hookworm and *S. mansoni* infections and between hookworm and *S. haematobium* infections. However, these findings need further investigation to elucidate possible mechanisms and the role of possible confounders such as host age, socio-economic or environmental factors which could be associated with both infections.

- The effect of *S. mansoni* and *S. haematobium* on malaria infection: A negative association between malaria parasite density and schistosome (S. *mansoni* and *S. haematobium*) infections was observed. This association have also been observed by other studies and several explanations have been given including immunomodulation and cross-reactivity between malarial specific antibodies and schistosome antibodies. However, these explanations seem to be inconclusive. It is apparent that more studies are needed to elucidate possible mechanisms and confounders.

- This study has shown that *P. falciparum* and and *S. haematobium* infections as predictors of anaemia; *P. falciparum* and hookworm infections are predictors of splenomegaly and hepatosplenomegaly and hookworm and *S. haematobium* are predictors of urinary tract pathology. However, the mechanisms under which concurrent infections cause anaemia or organ pathology remains largely unknown. Further investigations are therefore needed to elucidate possible mechanisms underlying clinical and pathophyisiological consequences of co-infection in different epidemiological settings and age groups.

- Since the current strategy for schistosomiasis and STH control is morbidity control by regular treatment using antihelmintics drugs such as praziquantel and albendazole, studies should be carried out to determine the best strategy for praziquantel and albendazole treatment particularly in areas with higher transmission of both *S. mansoni*, STH and malaria where single dose annual treatment (particularly of *S. mansoni* using praziquantel) seems not effective . In such areas, double or triple treatments annually could be considered.

- Studies on the effect of anthelmintic treatment on malaria infection should be continued taking into account factors such as intensity of helminth infection, vector and environmental factors, seasonality of malaria transmission, host age, host genetics and socio-economic status. The anthelmintic interventions could be combined with interventions against malaria

such as periodic administration of antimalarial drugs to school children to assess the combined effect on malaria and anaemia. The current study showed no impact of treating helminth infections on malaria infection. Although there was an overall reduction in malaria prevalence, intensity and number of malaria attacks at the end of the intervention, it was unclear whether this reduction resulted from the anthelmintic intervention or from other causes. Further studies are therefore needed to evaluate the impact of treating helminth infections only or combined treatment of helminths and malaria on malaria infection and anaemia.

- Finaly, this study demonstrated that *S. haematobium* and hookworm co-infections and antihelmntic treatment caused increased production of PfSE-IgG3 indicating a positive influence on anti-*P. falciparum* immune responses. However, the protective or pathological role of this effect could not be immediately established. Further studies are therefore needed to elucidate the protective or pathological role of increased production of PfSE-IgG3 in schistosome and *P. falciparum* co-infected individuals.

References

Fallon, P. G. (2000). Immunopathology of schistosomiasis: a cautionary tale of mice and men. *Immunol Today,* **21** (1): 29-35.

Hillier, S. D., Booth, M., Muhangi, L., Nkurunziza, P.,Khihembo, M., Kahande, M., Sewankambo, M., Kizindo, R., Kiza, M., Kiwanga, M., Elliot, A. M. (2008). *Plasmodium falciparum* and helminth coi-infections in semiurban population of pregnant women in Uganda. *J. Infect. Dis*, **198**: 920-927.

Montenegro, S. M., Miranda, P., Mahanty, S., Abath, F. G., Teixeira, K. M., Coutinho, E. M., Brinkman, J., Gonçalves, I., Domingues, L. A., Domingues, A. L., Sher, A, Wynn, T. A (1999). Cytokine production in acute versus chronic human Schistosomiasis mansoni: the cross-regulatory role of interferon-gamma and interleukin-10 in the responses of peripheral blood mononuclear cells and splenocytes to parasite antigens. *J Infect Dis*, **179** (6): 1502-1514.

Mwatha, J. K., Kimani, G., Kamau, T., Mbugua, G. G., Ouma, J. H., Mumo, J., Fulford, A. J., Jones, F. M., Butterworth, A. E., Roberts, M. B., Dunne, D. W. (1998). High levels of TNF, soluble TNF receptors, soluble ICAM-1, and IFN-gamma, but low levels of IL-5, are associated with hepatosplenic disease in human schistosomiasis mansoni. *J Immunol*, **160** (4): 1992-1999.

Nacher, M., Singhasivanon, P., Yimsamran, S., Manibunyong, W., Thanyavanich, N., Wuthisen, R., Looareesuwan, S. (2002). Intestinal helminth infections are associated with increased incidence of *Plasmodium falciparum* malaria in Thailand. *J Parasitol,* **88** (1): 55-58.

Ouma, J. H., Vennervald, B. J., Butterworth, A. E. (2001). Morbidity in schistosomiasis: an update. *Trends Parasitol*, **17** (3): 117-118.

Sokhna, C., Le Hesran, J. Y., Mbaye, P. A., Akiana, J., Camara, P., Diop, M., Ly A., Druilhe, P. (2004). Increase of malaria attacks among children presenting concomitant infection by Schistosoma mansoni in Senegal. *Mal J*, **3**: 43.

Spiegel, A., Tall, A., Raphenon, G., Trape, J. F., Druilhe, P. (2003). Increased frequency of malaria attacks in subjects co-infected by intestinal worms and *Plasmodium falciparum* malaria. *Trans Roy Soc Trop Med Hyg,* **97** (2): 198-199.

Appendix I. Data collection form

A. General information

No.	Question	Response	Variable
1.	Date (dd/mm/yy)	|____|____|____|	date
2.	School/Village identification number	|_____|	village _id
3.	Village name	|_____|	vill_name
4.	Child identification number	|_____|	child_id
5.	Child name	|_____|	child_name
6.	Child grade: **0 = Nursery; 1 = grade one; 2 = Out of school; 3 = Others**	|_____|	grade
7.	Child sex: **1 = Male; 2 = Female**	|_____|	sex
8.	Child age (years)	|_____|	age

B. Child clinical record

No	Question	Response	Variable
9.	Height (cm)	|_____|	height
10	Weight (kg)	|_____|	weight
11.	Body temperature (^{0}C)	|_____|	temp
12.	Abdomen: **0 = Normal; 1 = Distended**	|_____|	abd_dist
13.	Liver tender?: **0 = No; 1 = Yes**	|_____|	lv_tndns
14.	Liver consistency: **0 = Not palpable 1 = Soft 2 = Firm 3 = Hard**	|_____|	lv_consst
15.	Liver MSL (cm): Normal = 0cm	|_____|	lv_msl

B. Child clinical record (Contd...)

No	Question	Response	Variable
16.	Liver MCL (cm): Normal = 0cm	|_____|	lv_mcl
17.	Spleen consistency: **0 = Not palpable** **1 = Soft** **2 = Firm** **3 = Hard**	|_____|	sp_consst
18.	Spleen MCL (cm): Normal = 0cm	|_____|	sp_mcl
19.	Spleen MAL (cm): Normal = 0cm	|_____|	sp_mcl
20.	Other findings: **0 = None** **1 = Ascites** **2 = Abd. Swelling** **3 = Umbilical collaterals** **4 = Scars** **5 = Umbilical hernia**	|_____|	ot_fndngs

C. Ultrasound examination for *S. mansoni*

No.	Question	Response	Variable
21.	Liver image pattern: **0 = A Normal** **1 = BO feather streaks** **2 = B1 flying saucers** **3 = B2 spider thickening** **4 = C1 peripheral rings** **5 = C2 pipe stems** **6 = D ruff** **7 = E patches** **8 = F birds claws**	|_____|	liv_ip
22.	Other liver abnormalities: **0 = None** **1 = Cirrhosis** **2 = Fatty liver** **3 = Other abnormality**: _____	|_____|	otlivabn1
23.	Liver PSL (cm)	|_____|	liverpsl
24.	Periportal thickening: **0 = No; 1 = Yes**	|_____|	prptthkng
25.	Portal vein diameter (mm)	|_____|	pveindmt
26.	Gallbladder wall thickness: **0 = Not detected (< 4mm)** **1 = detected (> 4mm)**	|_____|	gbldthkng
27.	Portal systemic collaterals: **0 = Not detected** **1 = detected**	|_____|	systcoll
28.	Type of portal systemic collaterals **1 = Splenic varices** **2 = Coronary vein ≥ 4mm** **3 = Others:** _____	|_____|	systcolltp
29.	Ascites: **0 = Not present** **1 = Present**	|_____|	ascites
30.	Spleen length (cm)	|_____|	sp_length

D. Ultrasound examination for *S. haematobium*

No.	Question	Response	Variable
31.	Urinary bladder shape: **0 = Normal (rectangular)** **1 = Round (distorted)**	|_____|	ub_shape
32.	Bladder wall irregularity (inner surface thickening ≤ 5mm) **0 = No** **1 = Focal** **2 = Multifocal/diffuse**	|_____|	ub_irrglrty
33.	Bladder wall thickening (≥5mm, ≤10mm): **0 = No** **1 = focal** **2 = Multifocal/diffuse**	|_____|	wall_thick
34.	Mass (>10mm): **0 = No; 1 = Single; 2 = Multiple** (*Do not record as wall irregularity or focal thickening at the same time*).	|_____|	mass
35.	Pseudo polyp: **0 = No; 1 = Single; 2 = Multiple** *(Do not record as wall irregularity, thickening or mass at the same time).*	|_____|	psdopolyp
36.	Number of pseudopolyps: (i.e. Give score = number of masses + 2 e.g. for 3 masses give score of 3+2 = 5): *(Do not record as wall irregularity, thickening or mass at the same time).*	|_____|	psdplpScore
37.	Right ureter: **0 = Not visualized** **1 = Dilated, visualized at proximal and/or distal 1/3** **2 = Grossly dilated and/or entirely visualized**	|_____|	r_ureter
38.	Left ureter: **0 = Not visualized** **1 = Dilated, visualized at proximal and/or distal 1/3** **2 = Grossly dilated and/or entirely visualized**	|_____|	l_ureter
39.	Right renal pelvis (if dilated, record only after voiding): **0 = Not dilated, fissure ≤ 1cm** **1 = Moderately dilated: Parenchyma thickness (1-sided) > 1cm** **2 = Marked hydronephrosis, parenchyma compressed (thickness < 1cm)**	|_____|	r_pelvis

D. Ultrasound examination for *S. Haematobium* (Contd...)

No.	Question	Response	Variable
40.	Left renal pelvis (if dilated, record only after voiding): **0 = Not dilated, fissure ≤ 1cm;** **1 = Moderately dilated: Parenchyma thickness (1-sided) > 1cm** **2 = Marked hydronephrosis, parenchyma compressed (thickness < 1cm)**	|_____|	l_pelvis

E. Laboratory examination form

No.	Question	Response	Variable
41.	Haemoglobin level (g/L)	|___|___|___|	hb
42.	Malaria parasitaemia (No./200WBC)	|___|___|___|	mps
43.	Hookworm egg count (Day1): Slide1 Slide2	|_____| |_____|	hw_eggs1 hw_eggs2
44.	Hookworm egg count (Day2): Slide1 Slide2	|_____| |_____|	hw_eggs3 hw_eggs4
45.	S. haematobium eggs/10ml urine (Day1) S. haematobium eggs/10ml urine (Day2)	|_____| |_____|	sh_eggs1 sh_eggs2
46.	S. mansoni egg count (Day1): Slide1 Slide2	|_____| |_____|	sm_eggs1 sm_eggs2
47.	S. mansoni egg count (Day2): Slide1 Slide2	|_____| |_____|	sm_eggs3 sm_eggs4
48.	Trichuris egg count (Day1): Slide1 Slide2	|_____| |_____|	trich_eggs1 trich_eggs2
49.	Trichuris egg count (Day2): Slide1 Slide2	|_____| |_____|	trich_eggs3 trich_eggs4
50.	Other spp (specify) (Day1): Slide1 Slide2	|_____| |_____|	otsp_eggs1 otsp_eggs2
51.	Other spp (specify) (Day2): Slide1 Slide2	|_____| |_____|	otsp_eggs3 otsp_eggs4

Appendix II: ELISA for antibodies against PfSE (Batch I)

Day 1:

- 12000µl Coating buffer + 92,0 µl PfSE
  100 µl/well
  Incubation: overnight at 4°C.

Day 2:

- Wash x 4
- Blocking buffer
  150µl/well
  Incubation: 1 hour at room temp.
- Remove Blocking buffer
- Controls and Samples
  100µl/well
  Incubation: overnight at 4°C.

Day 3:

- Wash x 4
- 12000µl PBS + 4µl IgG3
  100µl/well
  Incubation: 1 hour at room temp. (or overnight at 4°C)
- Wash x 4
- 12000µl PBS + 3µl Streptavidin
  100µl/well
  Incubation: 1 hour at room temp.
- Wash x 4
- 12000µl Reaction buffer
  100µl/well
  Incubation: 16 min. at room temp.
- Stop solution
  50µl/well
- Read at dual wavelength 490/595nm

Appendix III: ELISA for antibodies against PfSE (Batch I) Buffers and calculations

- Microtiterplates Greiner Highbinding part.no. 65 5061.

- PfSE Conc.: 1045,5 µg/ml.

  Working conc: 8µg/ml
  1045,5 / 8 = 130,7~ 130 x dilution
  1 plate ~ 12000 µl : 12000 /130= 92,3 ~92 µl

- Coating buffer 8,4g $NaHCO_3$
  3,56g Na_2CO_3
  pH 9,5
  Dest. water ad 1000ml (store at 4°C/use within 30days)

- PBS 100ml PBS buffer x 10 pH 7,4(Bie&Berntsen)
  900ml dest. water (store at 4°C/use within 30days)

- Washing buffer (PBS with 0,03%Tween 20)
  300ml PBS buffer x 10
  2700ml dest. Water
  900µl Tween 20 (store at roomtemp/use within 3days)

- Blocking solution (PBS with 0,05% Tween 20 and 1 % Marvel)
  50 ml PBS
  25 µl Tween 20
  0,5g Marvel
  Dissolve for 15min. at magnetic stirrer (use within 1 day)
  (enough for 2 plates)

- Dilution buffer (PBS with 0,1% Marvel)
  100 ml PBS
  0,1g Marvel
  Dissolve for 15min. at magnetic stirrer (use within 1 day)
  (enough for 1 plate)

- Sample dilution 995µl dil.buffer + 5µl serum
  (incl.neg.control)
  1:200

Appendix III. (Contd…)

Positive control 1:1600 — 7995µl dil.buffer + 5µl pos control

Blank — Dilution Buffer

- IgG3 — Biotinylated mouse anti-human monoclonal antibody (clone HP-6050)Sigma no. B3523
  Conc. ca 1,5 mg/ml.

  Working conc.: 0.5µg/ml
  1,5mg/ml ~1500µg/ml
  1500 / 0,5 = 3000 ~ 3000 x dilution
  1 plate ~ 12000 µl : 12000 /3000= 4µl

- Streptavidin — Streptavidin-Peroxydase Polymer
  Sigma no. S2438
  Conc. 1,0mg/ml.

  Working conc.: 4000 x dilution
  1 plate ~ 12000 µl : 12000 / 4000 = 3µl

- Substrate/ Reaction buffer — OPD Tablets, 2mg for ELISA
  DAKO no. S2045

  15 min. before use:
  12000µl destilled water + 4 tablets
  Wrap the tube in silver paper
  Just before use:
  Add 5 µl H_2O_2

- Stop solution — 0,5M H_2SO_4

Appendix IVa: Plate set up for *P. falciparum* specific IgG3 analysis (Plates 1-5, 27-38).

(St = Standard; neg = Negative control)

Plate No: Date:

	1	2	3	4	5	6	7	8	9	10	11	12
A	St 1											
B	St 1											
C	St 2											
D	St 3											
E	neg											
F	neg											
G	blank											
H	blank											

Dilutions:

St 1 1:1600 Neg 1:200
St 2 1:3200 Samples 1:200

Appendix IVb: Plate set up for *P. falciparum* specific IgG3 analysis (Plates 6-26).

(St = Standard; neg = Negative control)

Plate No: Date:

	1	2	3	4	5	6	7	8	9	10	11	12
A	**St 1**	**St 1**										
B	**St 2**	**St 2**										
C	**St 3**	**St 3**										
D	**St 4**	**St 4**										
E	**St 5**	**St 5**										
F	**St 6**	**St 6**										
G	**neg**	**neg**										
H	**blank**	**blank**										

Dilutions:

St 1 1:400 St 2 1:800 Samples 1:200
St 4 1:3200 St 5 1:6400
St 6 1:12800 Neg 1:200